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The Journal of Laboratory and Clinical Medicine

VOL. XIII ST. LOUIS, MO., DECEMBER, 1927 NO. 3

CLINICAL AND EXPERIMENTAL

THE EFFECTS OF THE INTRACUTANEOUS INJECTION OF EPINEPHRIN IN CHILDREN*

BY J. D. PILCHER, M.D., CLEVELAND, OHIO

IN THE course of a certain investigation an adult several times received a small dose of epinephrin intracutaneously (0.2 mg. or 0.2 c.c. of a 1:1000 solution). Following each injection he experienced the phenomena attributed to epinephrin toxicity, including a fine, rapid tremor of hands and fingers, especially noted in this instance on writing, a slight weakness in the knees, pallor of the skin on the hands and fingers, and a certain "awareness" scarcely amounting to the "anxiety state" noted in the literature. As the results from this small dose of epinephrin administered intracutaneously were unexpected, several control experiments were made in which the same individual received either 0.2 mg. of epinephrin or a blank solution causing a similar local sensation, the nature of the solution being unknown to the subject; in each case the subject, from the resulting phenomena, reported correctly whether or not epinephrin had been injected. Since very few observations on the rapidity of absorption from intracutaneous injections were found in the literature, it appeared advisable to record the relative effects of intracutaneous, subcutaneous, and intramuscular injections of epinephrin. The observations were made on children from a few months to twelve years of age, all of whom were free from cardiac or other circulatory or kidney diseases. Epinephrin is especially suitable for this purpose because its physical effects, while harmless in the dosage used, are so readily measured objectively, notably by the action on the blood pressure, the tremor and the blanching of the skin; and subjectively, by the anxiety state and the sense of fatigue.

Methods.—The epinephrin was injected into the skin of the upper part of the flexor surface of the forearm and into the subcutaneous and muscular

*From the Babies' and Children's Hospital and the Department of Pediatrics, Western Reserve University.

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tissues of the same region. All subjects, except a few infants who received 0.1 mg., received the standard dose of 0.2 mg. (0.2 c.c. of 1:1000 solution). Twenty-four hours at least elapsed between experiments. As a comparison of results of the different methods of administration of epinephrin is of real value only in the same subject (not in one subject as compared with another), this uniform dose suited the purpose as well as a dose by weight. The blood pressure was taken by the usual cuff method and recorded frequently during the first five minutes and then every five minutes until it returned to the normal. Control injections of 0.2 c.c. of 0.25 per cent tricresol solution in normal saline were made in each subject to learn whether fear of, or pain from, the injection had any influence on the blood pressure, and in but one case was any effect noted: a three-year-old negro boy's blood pressure rose from 112 to 134 mm. from fear of the injection, and to 155 mm. immediately after the injection but returned to normal within one minute. In subsequent injections of epinephrin no fear was manifested, and there was no immediate rise in blood pressure. It may be well to state that while an

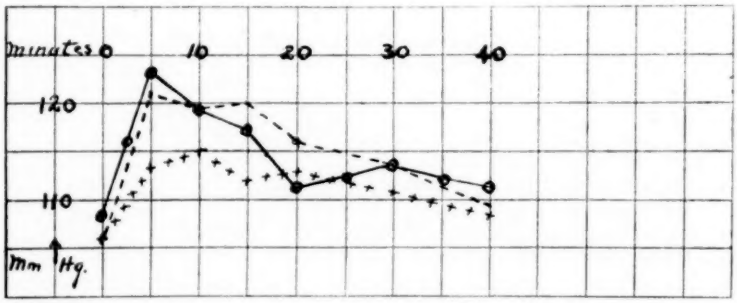

Fig. 1.—The average blood pressure records of all experiments.
o—o—o intracutaneous injection (22 experiments).
- - - - intramuscular injection (13 experiments).
+++++ subcutaneous injection (9 experiments).

occasional child protests against the first injection, there is seldom any objection to the subsequent ones; in fact it is not uncommon to have their associates ask to share their experience.

Effect on the Blood Pressure.—The average maximum systolic blood pressure rise is charted in Fig. 1. This maximum rise was usually reached within five minutes from the intracutaneous and intramuscular injection, occasionally somewhat later, and in about ten minutes by the subcutaneous route. The striking feature of the curve is that the average rise in pressure was somewhat greater by the intracutaneous than by the intramuscular or subcutaneous route. It is, of course, well known that epinephrin is fairly promptly absorbed from intramuscular injection and considerably more slowly from subcutaneous injection. The average maximum rise from the intracutaneous injections was 16 mm. (extremes, 4 and 38 mm.); by intramuscular injections, 15 mm. (extremes, 7 and 28 mm.), and by subcutaneous injection, 10 mm. (extremes, 0 and 26 mm.). The blood pressure was fairly well sustained above the normal, usually from forty to fifty minutes, occasionally somewhat longer. The differences between the intracutaneous and intramuscular routes are im-

material and a somewhat larger series of experiments might have shown the same effects; however, the point to be emphasized is that absorption by intracutaneous injection may be very rapid and considerable, at least equalling that of the intramuscular route. The effect on the blood pressure from intracutaneous injection may be very rapid for in those experiments in which it was measured in the first minute or two after the injection, the maximum rise occurred in this period; in 6 of 8 injections the rise was from 3 to 21 mm. in two minutes, and in 12 of 14 injections the rise was from 4 to 40 mm. in two minutes. Such a rapid rise may occur also from the intramuscular route but very seldom from the subcutaneous. Fig. 2 illustrates the effect on blood pressure in a nine-year-old girl who reacted well to all methods of injection. Fig. 3 is an example in which the blood pressure rose much more from the intracutaneous injection than from the intramuscular or subcutaneous route.

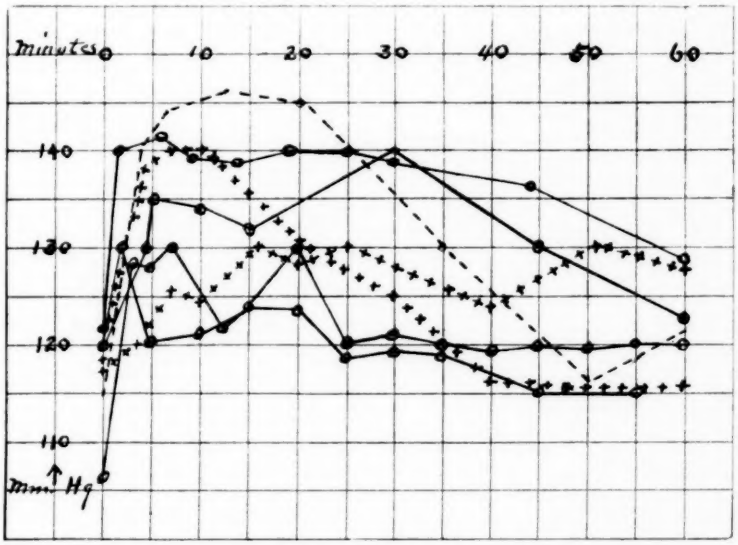

Fig. 2.—Blood pressure record in a 9-year-old girl; dose 0.2 mg. epinephrin.
o—o—o intracutaneous.
- - - - intramuscular.
+ + + + + subcutaneous.
The reaction to subcutaneous injection was unusually large.

The subjective and objective symptoms were in most patients roughly proportional to the vascular effect; i.e., they were the more pronounced the higher the blood pressure. However, there were exceptions, either a considerable rise in pressure without subjective symptoms or vice versa. The subjective symptoms and the tremors usually terminated before the blood pressure returned to normal; often they ceased a considerable time before this, but occasionally persisted from thirty-five to forty minutes. As the patients were young children the subjective symptoms were not always clearly expressed. When present they consisted of slight dizziness or headache or such phrases as "a pounding inside" (cardiac palpitation), "the bed wiggles" (tremor), or "I don't know what makes me so tired." The objective signs which never amounted to distress were tachycardia and usually a distinct

pallor of the extremities and face which disappeared before the blood pressure became normal. The tremor of hand and fingers was often best noted by the observer placing his fingers lightly on the flexor tendons at the wrist of the subject. Pallor was seen in several older infants from the intracutaneous injection of 0.1 c.c. of epinephrin solution; in these the blood pressure was not taken. The blanching of the skin is a very striking phenomenon in infants in whom tremor, such as occurs in older children or in adults, has not been seen.

A summary of the results obtained in one subject as an example of a marked reaction by all methods of administration of epinephrin follows: A white female aged nine years, weight 51 pounds; convalescent chorea; no cardiac lesion; a well-nourished, quiet, nonexcitable, well-behaved child. Two

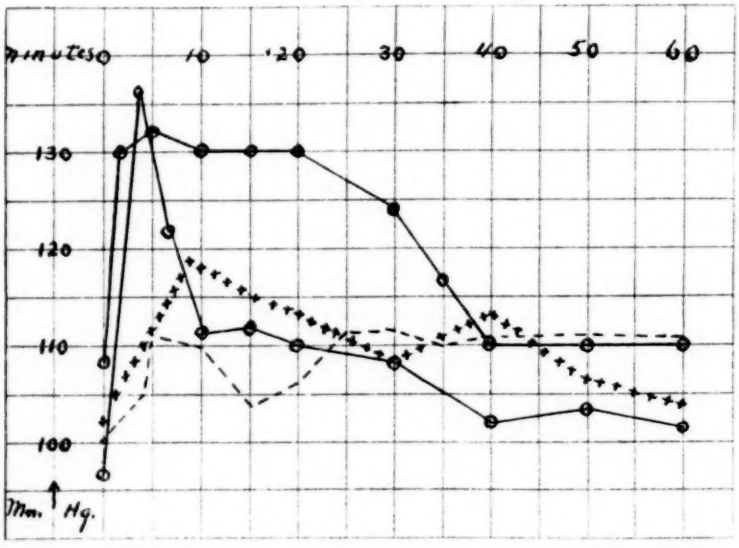

Fig. 3.—Blood pressure record in a 12-year-old boy; dose 0.3 mg. epinephrin.
o—o—o intracutaneous.
- - - - intramuscular.
+++++ subcutaneous.
The intramuscular reaction was less than the average in this instance.

minutes after an intracutaneous injection of 0.2 mg. of epinephrin the blood pressure rose from 120 to 140 mm. and remained about this level during forty minutes; the heart rate increased from 78 to 104 and shortly to 120 per minute and remained at this rate for forty minutes. Within four minutes a pronounced, fine, rapid, uncontrollable tremor of the hands was present, accompanied by pallor of the skin, extremities and face, and to a less extent of the body; the child remarked that "there is a pounding inside and my head feels funny" but continued to be happy and smiling. These signs and symptoms persisted, with short periods of decreased severity, for about forty minutes. The effects were similar in a second experiment but somewhat less in two others. From a single intramuscular injection the effect was as prompt and marked as from the intracutaneous injection above quoted. One of two

subcutaneous injections also gave about as prompt and marked a reaction as the intracutaneous and intramuscular but of less duration, while the other was less marked. This was the most marked reaction seen for the subcutaneous injections which, as shown elsewhere, were usually less than with the other methods of injection. The effect on the blood pressure is charted in Fig. 2.

DISCUSSION

The prompt action of epinephrin when injected intracutaneously is probably due to its rapid absorption. This may result from its being absorbed in relatively concentrated solution before local vasoconstriction limits absorption, the latter factor having been stated as the reason for the slight action from subcutaneous injection. Considerable pressure is exerted in making an intradermal injection and it is possible that because of this the solution gets into the venous or lymph channels of the skin readily. The pressure of the injection is considered to be a factor partly because a prompt rise in blood pressure follows the injection of epinephrin into the nasal submucosa under considerable pressure.[1]

Vollmer[2] noted certain changes in metabolism in infants and children following the intracutaneous injection of 0.1 c.c. of normal saline solution and attributed this to an influence acting through the nerve paths, for the changes were the same when the circulation above the injection site was occluded. The epinephrin effects here reported were not due to a local action on the nerve mechanism, for occlusion of the circulation by pressure on the arm prevented the action of intradermal epinephrin injection in the forearm. Such occlusion was maintained for periods of eight or nine minutes after the epinephrin injection in two subjects, and the blood pressure was unaffected, but release of the occlusion was followed shortly by a rise in blood pressure, which did not attain the usual level, however.

It was not feasible in the present work to determine the rate of absorption by injection of dyes or other substances into the skin to learn the time of appearance in the urine for this would have necessitated catheterization; nor would the presence of dyes in the vessels leading from the injection site have been evidence of absorption into the general circulation, for while pale streaks, due to constriction of the superficial vessels, sometimes two or three inches long, are often seen extending upward from the injection site, such phenomena have been observed without any subsequent demonstrable effect of epinephrin on the general circulation.

Langstein and Vollmer[3] and Levai[4] have noted that intracutaneous injection of epinephrin causes a rise in blood pressure but give no data.

No therapeutic application is suggested for the facts herein presented. Possibly in urgent need epinephrin would be more serviceable when introduced intracutaneously than intramuscularly, if the intravenous method could not be used. Because of the greater local discomfort associated with intracutaneous injections the method offers no advantages over the intramuscular route for general use.

CONCLUSIONS

The intracutaneous injection of epinephrin in human subjects is usually followed promptly by the systemic effects of the drug. The action is much more pronounced than with the same quantity administered subcutaneously and probably somewhat greater than by the intramuscular administration.

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THE EFFECT OF DRUGS ON THE NUMBER OF CIRCULATING WHITE BLOOD CELLS*†

By Walter J. R. Camp, M.S., M.D., Chicago, Ill.

SINCE the time of Hirt[11] the effect of drugs on the number of leucocytes has been extensively studied. The lack of agreement among the many investigators has led me to study the question with special consideration of the influence of the autonomic system on the distribution of white blood cells.

After a review of the extensive literature, I believe that the reasons for the contrary findings is due for the most part to lack of standardized technic. Space does not permit quoting the various results obtained by workers and for references one is referred to articles, such as that of Gehrig.[5] One finding of importance is the fact that no author has reported the finding of new cells in the blood stream of normal animals after the injection of these drugs. The changes occur so quickly (five minutes after injection) that it is difficult to see how new cells could be formed. Hence, the alteration produced is merely a shifting of cells from one part of the blood stream to another.

The fact that the blood cells are not in direct contact with the nerve fibers of the autonomic system does not prohibit them from being under the control of these nerves. The blood differs from other tissue in that it has no confining framework. It is well known that muscle and gland cells alter in structure after stimulation of their nerves, and in the case of the lymphoid tissue, cells are constantly leaving the base of supply. This process also is under nerve control. I believe it to be in harmony with these facts to consider that white blood cells may be under the control of the autonomic nervous system.

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*An abstract of a thesis submitted in partial fulfillment for the degree of Doctor of Philosophy in Pharmacology in the Graduate School of the University of Illinois, 1927.

†Department of Pharmacology, University of Illinois, Chicago, Illinois.

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METHODS

Only rabbits were used. They were kept on a diet of oats, cabbage, carrots, bread, and hay and were used at various times after feeding to eliminate the factor of digestion leucocytosis.

The usual method for counting blood cells was used. Only standardized instruments were used. Hayem's solution was used for counting the erythrocytes, and 1 per cent acetic acid colored with gentian violet was used for enumerating the leucocytes. Cover-glass preparations were treated with Wright's stain, and for the differential counts from 500 to 600 cells were counted. The cells were grouped as follows: neutrophiles (pseudoeosinophiles) lymphocytes, true eosinophiles, basophiles, monocytes, and myelocytes. For a detailed description of these cells reference may be made to the atlas of Kleineberger and Carl.[12]

Hemoglobin was determined by the Tallqvist scale. This is notoriously inaccurate but most closely approximated the color of rabbit hemoglobin. In view of the fact that accurate quantitative figures were not looked for, the desire being merely to determine whether or not the hemoglobin changed, I feel that this method answered the purpose. In no experiment was any marked or definite change found.

The red cells were counted in order to determine whether or not there was a change in blood concentration. In view of the fact that the greatest change obtained was a 17 per cent increase, the average being about 8 per cent, and that the change in erythrocyte count did not always occur at the same time as the leucocyte count, this procedure was not followed out in the majority of the experiments.

All drugs were injected subcutaneously.

Spleens were removed under aseptic conditions. It is realized that even after removal of the spleen much lymphoid tissue remains. However, this procedure is necessary to determine the rôle played by the spleen in drug leucocyte change.

EXPERIMENTS

Lack of space prevents the reporting of the experiments in detail, only summary tables being included. The net maximum figures reported are the percentage changes above or below the absolute total leucocyte count change.

Protocol I

Summary of Adrenalin Experiments With Fed Animals

Experiment No.	Net maximum changes						
	1	2	3	4	5	6	7
					Splenectomized		
Neutrophile	+118.16%	+ 58.68%	+102.05%	+124.80%	+106.09%	+ 49.25%	+76.00%
Lymphocyte	− 80.34	+ 10.39	− 80.52	−139.17	− 36.43	− 44.20	−50.90
Eosinophile	− 19.26	−106.57	+ 63.23	+155.42	+120.71	+ 93.95	+34.77
Basophile	− 62.05	− 93.22	− 15.09	+122.67	+ 56.10	+193.39	−15.86
Monocyte	− 31.93	−112.85	+131.79	+ 26.42	− 23.93	− 33.38	−50.90
	− 78.97						−90.90
Myelocyte			− 56.32	+663.92			

Protocol II

Summary of Eserine-Adrenalin Experiments

Experiment No.	Net maximum changes 1	2
Neutrophile	− 24.35%	− 39.65%
Lymphocyte	+ 32.84	+ 44.25
Eosinophile	−130.99	+ 39.85
Basophile	−126.43	− 5.65
Monocyte	+ 41.13	−116.95

Protocol III

Summary of Pilocarpine Experiments

Experiment No.	Net maximum changes 1	2	3	4	5	6	7
					Splenectomized		
Neutrophile	−54.27%	− 62.23%	− 48.77%	−131.36%	−87.53%	−132.78%	− 29.57%
Lymphocyte	+12.64	+ 66.05	+ 56.90	+186.91	+57.98	+210.45	+ 41.99
Eosinophile	−24.85	− 44.95	+ 54.32	−408.28	−99.99	− 0.33	+ 19.43
Basophile	−50.28	+124.19	−159.32		−50.41	+857.52	+575.89
Monocyte	+50.92	−229.02	+ 46.87	−182.25	+56.98	+ 10.27	+ 90.32
Myelocyte			−113.87	−105.38		−283.78	− 32.83

Protocol IV

Summary of Eserine Experiments

Experiment No.	Net maximum changes 1	2	
		1st phase	2nd phase
Neutrophile	−192.14%	−42.00%	+35.88%
Lymphocyte	+218.15	+73.30	−39.83
Eosinophile	+ 65.65	+20.60	−37.66
Basophile	+ 52.50	−13.49	−49.48
Monocyte	−219.35	−26.45	+40.79

Protocol V

Summary of Atropine Experiments

Experiment No.	Net maximum changes 1	2	3	4	5	6
					Splenectomized	
Neutrophile	+12.98%	+107.93%	+ 21.22%	+ 64.04%	+27.78%	+105.35%
Lymphocyte	− 5.84	− 19.48	− 20.82	− 46.63 −137.99	+14.37	− 87.94
Eosinophile	−55.78	+ 7.55	+104.09	−133.63	+27.99	+ 38.66
Basophile	+90.65	− 48.20	+ 14.24	+ 28.74	+48.02	+ 12.63
Monocyte	−27.80	+ 00.05	− 15.05	−137.76	+45.95	−149.73

Protocol VI

Summary of Acetylcholine Experiments

Experiment No.	Net maximum changes 1	2
Neutrophile	− 381.73%	−117.23%
Lymphocyte	+ 326.34	+158.67
Eosinophile	+ 427.66	+ 13.36
Basophile	+1275.72	− 28.84
Monocyte	− 326.34	−111.22

DISCUSSION

Two types of blood pictures may be recognized in the preceding tables: (1) parasympathetic, as produced by such drugs as acetylcholine, tartrates and oxalates, arecoline, pilocarpine, guanidine, eserine, and adrenalin after eserine; and (2) sympathetic, as effected by atropine, calcium, and adrenalin. Atropine and calcium may be said to produce a negative parasympathetic picture, since they depress parasympathetic nerves.

Each picture presents certain primary characteristics and a number of secondary features which are less constant.

PROTOCOL VII

SUMMARY OF ARECOLINE EXPERIMENTS

EXPERIMENT NO.	NET MAXIMUM CHANGES 1	2
Neutrophile	−16.93%	−55.41%
Lymphocyte	+46.62	+47.96
Eosinophile	+25.04	+80.68
Basophile	− 6.93	+ 5.23
Monocyte	−56.26	−93.89

PROTOCOL VIII

SUMMARY OF GUANIDINE EXPERIMENTS

EXPERIMENT NO.	NET MAXIMUM CHANGES			
	PHASE 1		PHASE 2	
	1	2	1	2
Neutrophile	−26.62%	+ 9.89%	+46.81%	+80.10%
Lymphocyte	+16.05	+27.28	−36.99	−30.69
Eosinophile	+59.89	+49.06	+34.87	−23.41
Basophile	− 4.91	− 3.53	+ 9.81	−11.39
Monocyte	+30.65	−14.39	−15.25	−19.19

PROTOCOL IX

SUMMARY OF CALCIUM EXPERIMENTS

EXPERIMENT NO.	NET MAXIMUM CHANGES 1	2	3
Neutrophile	+43.30%	+20.27%	+20.59%
Lymphocyte	−19.88	−45.82	+41.71
Eosinophile	+37.00	+43.31	−48.34
Basophile	-----	+ 0.68	−28.88
Monocyte	−35.00	−47.12	+63.13

PROTOCOL X

SUMMARY OF TARTRATE AND OXALATE EXPERIMENTS

EXPERIMENT NO.	NET MINIMUM CHANGES		
	TARTRATE 1	OXALATE 2	OXALATE 3
Neutrophyle	− 55.81%	− 32.27%	− 22.74%
Lymphocyte	+155.79	+ 26.39	+ 23.60
Eosinophile	+ 52.38	+151.94	+473.04
Basophile	+ 35.93	+ 70.07	− 9.58
Monocyte	−115.37	− 68.26	+229.36

PARASYMPATHETIC PICTURE

The striking portion of this picture is a primary marked increase in the lymphocytes. On examining the individual protocols, it will be seen that there is a tendency to a secondary increase in the neutrophiles and a corresponding decrease in the lymphocytes, i.e., a biphasic reaction. This, I am inclined to believe, is merely the result of fatigue of the nerves, and the result of an attempt to establish a normal balanced autonomic tone, in the attempt to do which there occurs excessive action just as in the healing of a wound there is excess production of tissue. It will also be seen from a study of the relative percentages that the increase in lymphocytes is, for the most part, at the expense of the neutrophiles and vice versa.

With the exception of the tartrates and oxalates, which produce an activation of the parasympathetics by withdrawal of the calcium, all the other substances effect the change by directly influencing the peripheral parasympathetic apparatus, i.e., myoneural junctions or nerve endings as the case may be, either by sensitizing, as with eserine, or by stimulating, as with pilocarpine or adrenalin after eserine, and the other drugs.

TABLE I

THE CHANGE IN PARTITION OF LEUCOCYTES EFFECTED BY DRUGS INFLUENCING THE PARASYMPATHETIC NERVES

DRUGS USED	1	2	3	4	5 PHASE A	5 PHASE B	6 PHASE A	6 PHASE B	7
Neutrophile	−100%	−100 %	−100%	−100%	− 50% + 50%	+100%	−100%	+100%	−100%
Lymphocyte	+100	+100	+100	+100	+100	−100	+100	−100	+100
Eosinophile	+100	+100	+100	− 71.43 + 28.57	+100	+ 50 − 50	+100	−100	− 50 + 50
Basophile	+ 50 − 50	+ 66.67 − 33.33	− 50 + 50	− 50 + 50	−100	+ 50 − 50	+ 50 − 50	−100	−100
Monocyte	−100	− 66.67 + 33.33	−100	+ 71.43 − 28.57	+ 50 − 50	−100	−100	+100	+ 50 − 50

1 = Acetylcholine.
2 = Tartrates and oxalates.
3 = Arecoline.
4 = Pilocarpine.
5 = Guandine.
6 = Eserine.
7 = Eserine + Adrenalin.
+ = Increase.
− = Decrease.
Percentage represents portion of total experiments.

TABLE II

THE CHANGE IN PARTITION OF LEUCOCYTES EFFECTED BY DRUGS INFLUENCING SYMPATHETIC NERVES OR DEPRESSING PARASYMPATHETIC NERVES

	ADRENALIN	ATROPINE	CALCIUM
Neutrophile	+100 %	+100 %	+100 %
Lymphocyte	− 85.71 + 14.29	− 85.71 + 14.29	−100
Eosinophile	+ 71.43 − 28.57	+ 57.14 − 42.86	+ 66.67 − 33.33
Basophile	+ 57.14 − 42.86	+ 85.71 − 14.29	− 66.67 + 33.33
Monocyte	− 71.43 + 28.57	− 57.14 + 42.86	− 66.67 + 33.33

+ = Increase.
− = Decrease.
Percentage represents portion of total experiments.

The lymphocytes are increased in 100 per cent of the experiments, while the neutrophiles are decreased in 93 per cent and increased in 7 per cent of the cases.

The change in the number of other cells is less constant. Eosinophiles are increased in 82.65 per cent of the experiments and decreased in 17.35 per cent. Monocytes are decreased in 70.75 per cent and increased in 24.25 per cent of the experiments; while the basophiles are increased in 69.05 per cent and decreased in 30.95 per cent of the experiments.

TABLE III

	INCREASE	DECREASE
Neutrophile	7.00%	93.00%
Lymphocyte	100.00	
Eosinophile	82.65	17.35
Basophile	69.05	30.95
Monocyte	24.25	76.75

SYMPATHETIC PICTURE

The sympathetic picture is somewhat different in that neutrophiles increased in 100 per cent of the experiments, while the lymphocytes decreased in 90.47 per cent. These findings are just the reverse of those in the parasympathetic picture and are characteristic. Secondarily, there is an increase in the eosinophiles, basophiles and monocytes in about two-thirds of the experiments. Table IV summarizes these findings.

TABLE IV

	INCREASE	DECREASE
Neutrophile	100.00%	0.00%
Lymphocyte	9.53	90.47
Eosinophile	65.08	34.92
Basophile	69.84	30.16
Monocyte	65.08	34.92

Analysis of Tables III and IV and the individual protocols shows that preponderance of lymphocytes and a corresponding decrease in the neutrophiles is characteristic of the blood picture produced by parasympathetic drugs, while a preponderance of neutrophilic leucocytosis and a corresponding decrease in the lymphocytes is indicative of the action of the sympathetic drugs.

As can be seen from a review of the literature the fact that drugs are capable of disturbing the normal partition and distribution of leucocytes in the circulating blood has long been known and quite intensively investigated. Nevertheless there is some disagreement as to just what changes take place and what mechanism is involved.

Hess[10] and Hatiegan[8] believe that concentration of the blood affects the change in the white count with adrenalin. In my own experiments the change in red cells has never been greater than plus or minus 17 per cent and, further, the greatest change in the number of leucocytes does not always occur where the red cell change is greatest. It is quite difficult to see why concen-

tration of blood following the use of one drug effects a lymphocytosis, while following another effects a neutrophilia. Early in this work, I dropped this factor as a consideration because of lack of any great change in the number of erythrocytes. It may be added that following the injection of pilocarpine the blood flows more rapidly than normal and that the clotting time is lengthened considerably. The opposite is true of atropine.

Harvey,[7] Rous,[17] Dixon,[4] Dazzi,[3] and Oehme[16] consider the contraction of the spleen the cause of the increase of white cells in the circulation. Aschoff,[1] Billigheimer,[2] Grimm,[6] and Kreuter[13] state that contraction of the spleen is not sufficient to explain the changes. My own work seems to rule out the spleen conclusively, since virtually the same reaction was found after splenectomy as before, as can be seen from the tables. I conclude then that the spleen is of little, if any, importance in effecting drug leucocytosis.

Abel, quoted by Grimm, concluded that the hyperglycemia effected by adrenalin was the causative factor. While it is true that adrenalin produces a hyperglycemia (Sollmann, p. 442), some other mechanism must be considered in the production of pilocarpine leucocytosis, for McGuigan[15] found that pilocarpine effected no change in glycogenolysis. Atropine also effects no change in sugar metabolism, although the differential count after this drug simulates that produced by adrenalin. However, a leucopenia is produced by atropine. Levine and Kalais[14] determined the effect of insulin on the blood picture and found an increase in red and white cells, the latter being the greater. In view of the fact that pilocarpine effects a leucocytosis and atropine simulates adrenalin in the type of action without either drug influencing sugar metabolism, and the fact that insulin also increases the number of leucocytes while decreasing the amount of sugar, I conclude that a consideration of the factor is not imperative.

As has been mentioned before, some authors believed that the autonomic nervous system had something to do with the partition of leucocytes in the blood, but I do not believe any one has offered conclusive evidence. I feel that the blood may react in two ways to the injection of drugs: First, the production of a sympathetic picture characterized by an increase in the neutrophiles, by a decrease in the lymphocytes, and by less constant increase in the other myeloid cells. This change may be effected either by increasing the activity of the sympathetic nervous system, as with adrenalin, or decreasing the parasympathetic nerves and leaving the tone of the orthosympathetics undisturbed as with atropine or calcium; second, the production of a parasympathetic picture characterized by an increase in the lymphocytes and a decrease in the neutrophiles. The change in the other cells is less constant. We feel that the production of a parasympathetic, or in other words, a *negative* sympathetic picture, will account for this. I feel that increasing the parasympathetic activity does not necessarily decrease the sympathetic tone for all cells.

The parasympathetic action may be effected by pilocarpine, eserine, arecoline, tartrates, and oxalates, which amounts to withdrawal of calcium, and of adrenalin after eserine. In some previous work,[9] I have shown that adrenalin after eserine is capable of acting on the parasympathetic nerves when

given in small doses. The fact that adrenalin after a sensitizing dose of eserine, that is, a dose which in itself will effect no demonstrable change, brings about a typical parasympathetic blood picture, makes me believe that this is conclusive evidence that the distribution and partition of leucocytes in the circulating peripheral blood are mediated through and are under the control of the autonomic nervous system. I further believe that the blood must be considered as a tissue and, as such, its function and activity must be under nervous control.

Whether this action is direct or mediated through the control of inorganic ions, such as calcium and potassium, I am not ready to draw absolute conclusion, but on the evidence of the calcium, tartrates, oxalates, and guanidine experiments, I believe such is the case. It is probable that this factor enters into the action of these nerves on all organs.

I offer the following reasons why I believe that the distribution of leucocytes in the peripheral blood is under the control of the autonomic nervous system:

1. The same blood picture is produced by such chemically different substances as pilocarpine, tartrates, eserine, arecoline, guanidine, and adrenalin after a sensitizing dose of eserine.

2. The same differential blood picture is effected by substances stimulating the sympathetic nervous system as adrenalin and by such substances as decrease the activity of the parasympathetic nerves as atropine and calcium.

3. The pilocarpine action on the blood is promptly and completely checked by atropine just as pilocarpine action on any other organ is stopped by this same drug, and further, pilocarpine is ineffective after atropine has been given.

CONCLUSIONS

1. The distribution of leucocytes in the peripheral circulating blood of rabbits is controlled by the autonomic nervous system.

2. The partition of leucocytes in the peripheral circulating blood is an index of autonomic balance, the lymphocytes running parallel to parasympathetic tone and the neutrophiles to sympathetic tone.

3. The increase of neutrophiles takes place at the expense of the lymphocytes and the lymphocytes increase at the expense of the neutrophiles.

4. With an increase in sympathetic tone there occurs a concomitant decrease in the lymphocytes.

5. With an increase in the parasympathetic tone there occurs a concomitant decrease in the neutrophiles.

6. The spleen plays no part in the production of a drug leucocytosis.

7. It is possible and probable that this influence of the autonomic system is mediated through the medium of inorganic ions, such as calcium.

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A CRITIQUE OF THE LIPASE "PICTURE" METHOD*

By Harry Beckman, M.D., Milwaukee, Wis.

FOR the past several years Falk and his collaborators have been studying the lipase activities of the extracts of various organs and tissues removed from recently killed animals. The method employed[1] is essentially as follows: the animal is killed and the organ is immediately removed and extracted with a definite quantity of water for a given period of time; portions of this extract are then incubated for a given time and at a given temperature with equi-molecular quantities of a number of esters, and the relative amounts of acid produced under these conditions are plotted graphically. This graph is then presented as a "picture" of the lipase activity of the particular structure which is being studied. Recently Falk[2] has stated his belief that these pictures, as compared with other methods of studying life phenomena, will perhaps "approach more closely from a chemical standpoint the properties and changes which occur in living organisms." After much disappointment with the method, I wish to record my reasons for believing that it perhaps has very little value at all.

1. *The Method Does Not Take Into Account the Serum Lipase.*—Rona and Michaelis[3] definitely showed, in 1911, that there is a serum lipase which differs in activity in the different animals. A little later Rona and Bien[4] were able to show that this lipase is not the pancreatic lipase in circulation by proving that the two differ in optimal reaction and that they are affected differently by salts. Also the pancreatic lipase acts on both homogeneous and heterogeneous systems while the serum lipase is effective on only a homogeneous system; however, this does not justify us in not taking it into account here, for in those cases in which the esters are soluble in the extracts we are dealing with homogeneity. Nor can we assume it to be a constant quantity, for if we range ten splenic extracts side by side we shall see that no two are of exactly the same shade of pinkish-straw, however careful we may

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be in our procedure and in the washing away of surface blood. That is to say, the blood content per gram of spleen varies and therefore these extracts contain varying amounts of serum lipase.

This serum lipase is not taken into account at all in the method, but it is assumed that all of the lipase activity observed is due solely to that lipase which is indigenous to the organ under investigation.

2. *The Method Does Not Take Into Account the Profound Disturbances of the Whole Organism at the Moment of Death.*—In unpublished experiments performed in this laboratory it was found that the subcutaneous injection of pilocarpine in the albino rat was apparently followed by an increased lipase activity of the spleen, as measured in terms of the activity of the spleen extract upon a number of esters. However, when the normal lipase activity of the unstimulated organ was determined quantitatively in a large number of controls, it was found that these apparent pilocarpine increases were all well within the normal range, which was astonishingly wide. The methods and findings in these control experiments will be briefly presented here.

EXPERIMENTAL METHOD

a. Female albino rats of approximately the same age were used in groups of ten. Five animals of each group were killed by a crushing blow on the head; the other five were killed by the inhalation of concentrated ether fumes in a closed chamber.

b. Immediately after killing, the abdomen was opened and the spleen removed and weighed. Each individual spleen was then treated as follows: thoroughly pulped in a mortar without sand, washed into a flask with 100 c.c. of distilled water per gram of spleen, six drops of toluene added and the flask stoppered and placed in the refrigerator for twenty-two hours; removed from the refrigerator, filtered, 10 c.c. of the filtrate diluted to 200 c.c. with distilled water, the whole neutralized, and 25 c.c. of this diluted and neutralized filtrate incubated in a stoppered flask with 5 millequivalents of the following six esters: benzyl-acetate, methyl-acetate, methyl-butyrate, ethyl-benzoate, ethyl-acetate, and benzyl-benzoate. Toluene was added to each flask before placing it in the incubator.

c. The period of incubation in the presence of these substrates was twenty-two hours at 39° C. The amount of acid produced under these conditions, titrating with N/100 NaOH with phenolphthalein as indicator, was taken as the measure of the lipase activity of a particular spleen after deducting for the necessary blanks.

This is a modification to meet experimental conditions of the method employed by Falk, Noyes, and Sugiura.[1]

TABLE I

	BLOW GROUP					ETHER GROUP				
Rat No.	1	2	3	4	5	1	2	3	4	5
Rat weight in gm.	195	202	188	178	21[illegible]	206	196	198	197	212
Spleen weight in gm.	0.48	0.47	0.45	0.72	0.4[illegible]	0.46	0.56	0.58	0.48	0.65
Lipase activity in c.c. NaOH	2.15	1.10	1.70	1.45	0.9[illegible]	1.35	3.10	5.50	2.75	0.40

PRESENTATION OF FINDINGS

The findings in one group of ten animals, five killed by a blow and five by ether, are shown in Figs. 1 and 2 and in Table I. For purposes of simplicity only this one of many similar groups is presented here.

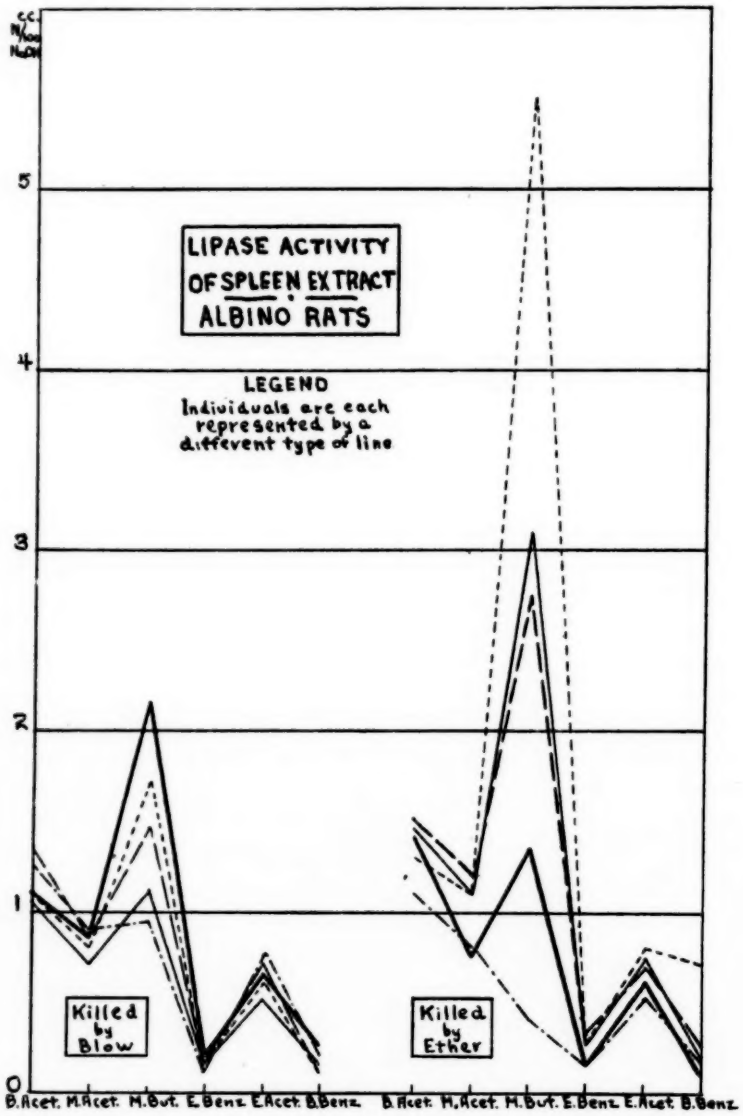

Fig. 1.

In Fig. 1 the equivalent amounts of acid produced, in corrected cubic centimeters of NaOH as determined by titration, are shown as ordinates; the esters are placed as abscissae. It will be seen that there is a certain characteristic "picture" for all the animals in the group killed by a blow, but that very considerable quantitative differences exist between individuals of the

group. In the group killed by ether the same type of "picture" is again seen, but the quantitative differences between individuals are even greater than in the blow group.

In Table I the weight of each of the ten animals of the group is stated, together with the weight of its spleen and the lipase activity of that spleen in terms of cubic centimeters of acid produced by its action on methyl-butyrate. That there is no constant relationship between the weight of the animal, the weight of its spleen, and the lipase activity of the spleen, is quite apparent.

In Fig. 2 the percentage variation from the average methyl-butyrate activity of these spleen extracts is shown. The data graphically presented in this figure were obtained as follows: the five figures representing the ac-

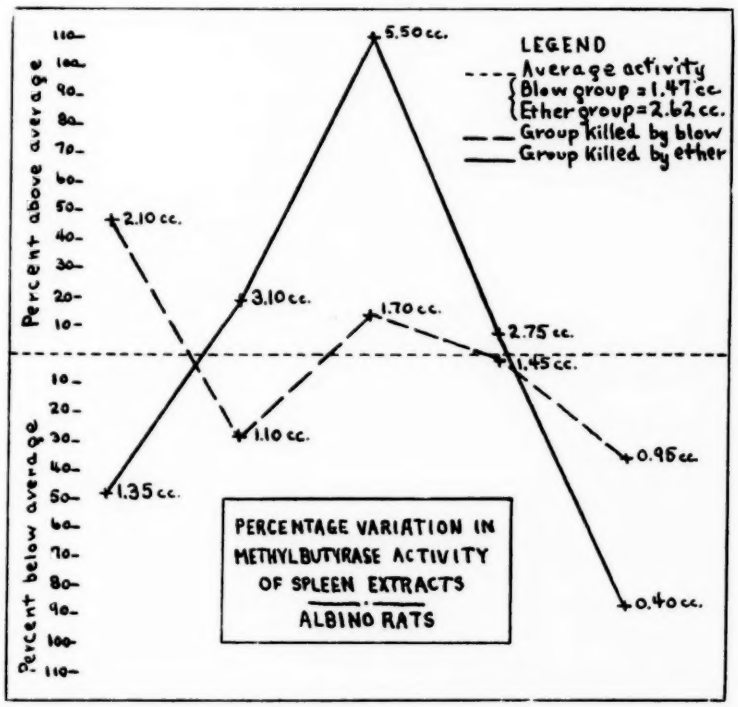

Fig. 2.

tivity of the blow group on this particular substrate (as shown in Table I) were added, and this sum divided by five in order to obtain the average. This average figure (1.47 c.c.) was then represented by the dotted line extending horizontally through the middle of the figure, and the number of cubic centimeters representing the activity of the individuals of the group were placed in relation to this line according to scale. Percentages were then placed as ordinates above and below the line. The individuals of the ether group were plotted in the same way, the average figure here being 2.62 c.c. It is shown in this figure that the five members of the blow group departed from the average of their methyl-butyrate activities as follows: one, 46.2 per cent above; one, 27.2 per cent below; one, 15.6 per cent above; one,

1.36 per cent below; and one, 35.3 per cent below. In the other group the individual variations were: one, 48.4 per cent below; one, 18.3 per cent above; one, 109.9 per cent above; one, 4.9 per cent above, and one, 84.7 per cent below.

Two ways of accounting for these great variations have occurred to me. They may represent the normal variation between the splenic lipase activity of individual animals, or they may be simply an index of the profound disturbance of all activities which takes place at death. If the former, then we

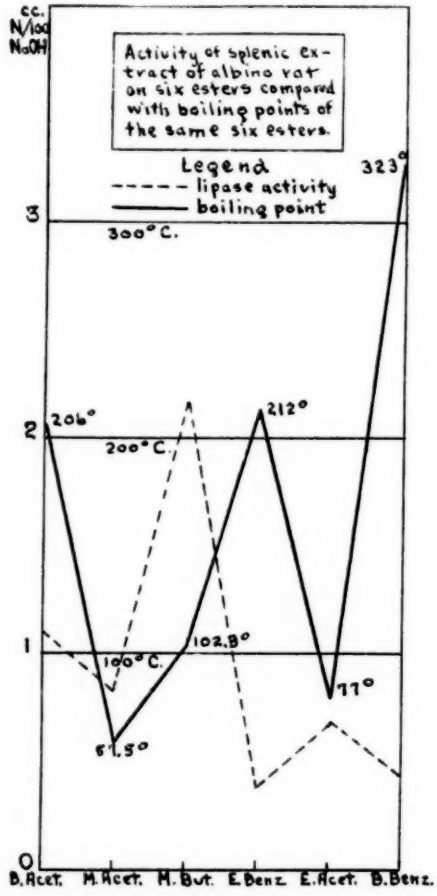

Fig. 3.

would seem to be entirely unwarranted in accepting any set of determinations, whether they be presented in the form of the absolute or the relative quantitative figures, as representing even the most remote approximation to the typical picture of an activity, for in the statistical approach to a biologic problem the "typical" is the "average"; and it would seem impossible to determine an acceptable average here. On the other hand, if these variations are merely an index of the profound functional disturbances at the moment of violently induced cessation of life, then we have a true "picture"

indeed, but it is only a picture of death. This latter would seem to me the more reasonable interpretation. That emotional disturbances cause considerable variations from the normal in many life processes is coming to be recognized more and more both in the laboratory and in clinical practice. Witness the scepticism of Barbour and Hamilton[5] who have recently said, in discussing their new method of determining the specific gravity of fluids, "In view of the well-established but neglected fact that relatively slight emotional disturbances are associated with anhydremia, one wonders how many really normal blood samples have ever been taken." Excitement increased the blood concentration in their dogs sometimes as much as 10 per cent. Must we not allow that the supreme emotional disturbance, which is death, may profoundly upset the enzyme activity of any organ or tissue?

3. *The Method Does Not Take Into Account the Escape of the Esters From Contact With the Extract.*—The assumption is made that in placing definite quantities of an organ extract in contact with equi-molecular quantities of a number of esters in separate tubes, and maintaining the mixture at a given temperature for a given time, the resultant amounts of acid found will be directly comparable; that is to say, we assume that the enzyme will be in contact with the same number of molecules in all of the tubes. And so it would be if all of the esters were of the same boiling point. But they are not, as is shown in Fig. 3 where the curve of lipase activity of a particular spleen extract upon six esters is superimposed upon the curve of the boiling points of these esters. Obviously substances of such wide range in boiling point will vary considerably in their rate of evaporation; exposing equal surfaces of the substances in calibrated tubes in the incubator for a given time will show the quite different rates at which they disappear. This being true, the amount of activity of the extract upon the different esters is quite a different thing from the "picture" of this activity presented in the figure. Leaving out of consideration four of the esters, which are not soluble under the conditions of these experiments, it must be evident from observation of this figure that the comparative activity of the extract upon the two soluble esters, methyl-acetate and methyl-butyrate, is not here shown for the reason that the methyl-acetate is eluding by evaporation the destroying grasp of the lipase at a much faster rate than is the methyl-butyrate.

This rate of escape of the various esters from the ester-extract mixtures is a factor not taken into account in the method. Indeed, if graphs, such as are presented by Falk and his collaborators and in this present paper are to be accepted as true pictures of the lipase activity of organs, then it would seem that a photograph of one's own hand with several fingers oscillating so rapidly as to appear only as blurs on the negative, might, with equal validity, be offered as a typical picture of the human presensile organ.

SUMMARY

The value of lipase "pictures" obtained by the method of Falk et al is brought into question for the following reasons, which are developed in the paper:

1. The method does not take into account the serum lipase.

2. The method does not take into account the profound disturbance of the whole organism at the moment of death.

3. The method does not take into account the escape of the esters from contact with the extract.

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HOUSEHOLD OBJECTS AS CAUSES OF HYPERSENSITIVENESS*

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THE most important factor contributing to the successful management of the hypersensitive individual is the ability to correlate the results of the diagnostic skin tests with the objects in the patient's environment. Often this is a very simple procedure. But, just as often, the association between diagnostic test and clinical exposure is a much more difficult problem to determine. In many instances the diagnostic test has no apparent relation to anything in the patient's environment. This is due almost entirely to our ignorance of, and disregard for, the relation between raw material and finished commodity.

It becomes evident, therefore, that in the practice of allergy it is highly important to keep constantly in mind the various objects with which the patient is prone to come in close proximity, and to have some working knowledge of the substances that enter into the composition of these objects. It was with this thought in mind that an investigation was begun to determine the composition of some of the most important commodities and to determine the various objects in which any particular raw product was used. The facts presented herewith were obtained from several sources: standard textbooks on manufacture, textiles, etc.; inquiry into commercial processes directly through the producer; United States Government Bureau Reports, and at times microscopic investigation. No attempt is made at this time to produce a complete outline of facts, but rather to present a few important illustrative reports with the idea that greater interest may be stimulated in the study of this important phase of allergy.

First of all, it is necessary to know in a general way what substances, capable of causing sensitization, are likely to enter into the make-up of some of our more important commodities, chiefly those of the home. Pillows are usually made of chicken, duck, or goose feathers, and sometimes of turkey or pigeon feathers. Cotton, kapok, silk floss, straw, or animal hairs are

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also used to stuff pillows. Mattresses are made of cotton, animal hairs, kapok, straw, and cotton or wool felt. Most blankets are made of cotton or wool, or both. Domestic rugs are made of cotton or wool; oriental rugs may contain also goat hair, camel hair, linen, and silk. Some rugs are also made of vegetable fibers. Furniture may be upholstered with cotton, wool, silk or linen material, or with mohair (goat hair); the furniture stuffing is either feathers, cotton, silk floss, animal hair, wood shavings, or vegetable fibers. The chief materials used for clothing are cotton, sheep wool, linen, or silk. Of recent years camel hair and goat hair have been taking a prominent part in the manufacture of clothing. It is a notorious fact that furs are very rarely purchased under their original names. Most furs on the market are clipped, sheared, dyed, and pulled in such a way as to resemble those which are more expensive. In cosmetics the ingredient giving the greatest amount of trouble as a sensitizing agent is orris root.

I will now proceed to enumerate more in detail the various raw materials and the manner in which they are used in the manufacture of finished articles. Many of the materials to be discussed have already been incriminated from time to time in the production of hypersensitiveness; others are still problematic, but are undoubtedly capable of producing hypersensitiveness; many of these undoubtedly do so, but we are unaccustomed to investigate them thoroughly.

ANIMAL HAIRS

The most important class of atopens is the group of animal epiderms, and of these the animal hairs play a substantial part. The animal hairs used for industrial purposes are sheep wool, various goat hairs, camel hair, cow hair, horse hair, rabbit hair, furs, and a few minor hairs, such as cat hair, hog hair, and deer hair.

Wool.—The wool of the sheep is the most important animal hair universally used. It is employed in the manufacture of many articles of clothing both alone and mixed with other materials. It is also used in various ways in the more or less raw condition. Hypersensitiveness to wool has been reported in the literature. In my own experience I have, on several occasions, obtained strongly positive skin reactions. Unspun wool is used for padding of robes and quilts, stuffing of mattresses, quilts, and sheepskin coats. Some of the better known materials for which wool is used are as follows:

Albatross	Wool gabardine	Mohair (imitation)
Astrakhan	Homespun	Tweed
Blankets	Jersey	Velour
Broadcloth	Mackinaw	Whipcord
Chinchilla (wool)	Melton	Padding for robes, quilts
Cravenette	Rugs	Wool for medical purposes
Doeskin	Serge	Mattress stuffing
Felt (wool)	Suede cloth	
Flannel	Tapestry	

There are many other materials and purposes for which wool is used, but it is not necessary to enumerate them here. It is also important to remember that there are several varieties of sheep from which the wool is ob-

tained, and that specificity as to species may exist as regards sensitization. Archer divides sheep into thirty-two varieties. According to Matthews[1] sheep may be classified into three groups:

1. Ovis aries, domestic sheep, comprising the most important breeds.
2. Ovis musmon, Mediterranean sheep.
3. Ovis ammon, the Argali, from which Asiatic wool is obtained.

Goat Hair.—Goat hair has been reported as being responsible for some cases of asthma, vasomotor rhinitis, and dermatitis. The hair of the goat chiefly used in industry is mohair, which is obtained from the Angora goat. This animal, originally in Persia, is now being bred extensively in Cape Colony, Australia, and in the United States, chiefly in Oregon, California, and Texas.[1] The chief uses for mohair are[1, 2]:

Plushes for furniture upholstery, and railway cars. Here the term "mohair" is often erroneously used for other fabrics.
Coat linings
Men's summer suits
Dress goods
Braids and wigs
Rugs
Portières
Skins with hair used for carriage robes, muffs, coats, and trimmings for coats and capes.

Cashmere is obtained from the Cashmere goat native to Tibet and the district of Kashmir in Northern India. This hair is used chiefly in the manufacture of Cashmere and Paisley shawls and Indian shawls. Most of the commercial fabrics designated by the term "cashmere" are various types of ordinary sheep wool.

Alpaca, or auchenia paco, a species of llama, a domestic goat of Peru, yields a fine fleece of several colors which is used chiefly for natural colored alpaca yarns in the hosiery trade. The name "alpaca" is also given to a variety of wool substitutes. "Gorilla yarn" is a complex mixture of such hair fibers as alpaca, sheep wool, mohair, and cotton and silk wastes.

Camel Hair.—This hair is now being popularized, especially in this country. It is usually employed in conjunction with other materials in the manufacture of fabrics. The Jäger cloth is a camel hair fabric. Coats and sweaters are frequently made of camel hair. Blankets, rugs, dress goods, linings, and beltings frequently contain camel hair; brushes are often made of camel hair. I have observed instances of camel hair hypersensitiveness.

Cow Hair.—Cattle hair is seldom used alone in fabrics on account of its short staple. It is found in coarse carpet yarns, blankets, and in a variety of cheap felted goods. It is used sometimes for the stuffing of mattresses and mixed in building materials, and enters into the manufacture of some animal toys. Positive skin reactions to cattle hair protein are rather common in my experience, although it is not so easy to interpret its clinical significance in every case.

Horse Hair.—This is used chiefly as stuffing materials for furniture and other upholstery. It is also used in mattresses and as padding for coats.

Rabbit Hair.—This hair is very frequently employed in commerce although this fact is very seldom appreciated. Rabbit hair is the most common hair used in the manufacture of felt for hats. It is also found in other felts. In some parts of this country it is extensively employed as stuffing of pillows and even mattresses. Rabbit fur is also used in the lining of gloves, and on cuffs and collars. It is also of vast importance to realize that rabbit fur is very commonly altered by shearing and dyeing and sold under names of better furs, such as sable, seal, chinchilla, fox, ermine, electric seal, and Hudson Bay seal.

Other Hairs.—It is possible that some of the hairs of lesser importance may play an occasional rôle in causing hypersensitiveness. Badger hair is often used for shaving brushes. Mule hair is sometimes employed for the better variety of floor brushes. Various types of brushes are also made of hog hair.

FEATHERS

Chicken, duck, and goose feathers are the most common feathers used in large quantities, and constitute one of the greatest causes of allergic disease. These feathers find their greatest use in pillows, and among foreign-born peoples the use of feather beds is an additional source of trouble. Turkey, and occasionally, pigeon feathers are also used in pillows. Hats are often trimmed with clipped duck or goose feathers. Down is employed in the stuffing of furniture and sometimes in quilts. Ostrich feathers are used on hats and as a trimming for women's clothing; marabou, the feathers of a species of African stork by the same name, is used in the trimming of women's and infant's clothing. Egret, or aigrette, feathers for headdress, obtained from various species of herons, are a possible cause of hypersensitiveness.

SILK

Silk, the fiber spun by certain species of caterpillars, is, of course, of animal origin. Hypersensitiveness to silk has been known to cause various manifestations of allergy. The silk fibers are twisted together to form threads which are used in weaving silk cloth. Broken, loose, and tangled fibers, the so-called silk floss or waste silk, are used for spinning purposes. It is important to realize that many articles and fabrics are sold under names suggestive of silk, but which contain only a small proportion of silk or none at all. The most important articles made of silk are waists, shirts, dresses, underwear, hosiery, thread, silk floss for pillows, etc., rugs and upholstery. The following are some of the most common uses of silk[3]:

Broadcloth (silk)	Pongee
Brocade	Rugs
Canton crepe	Silk poplin
Silk casement cloth	Radium silk
Chiffon	Wash satin
China silk	Tub silk
Crepe de chine	Tulle
Duchess satin	Thread
Foulard	Upholstery and tapestries

Faille	Velvets
Georgette	Silk floss for lining, knit goods, hosiery, mufflers, cheap silk neckties, pillows, etc.
Heavy taffeta	
Jersey	
Plush	

Some products containing no silk but sold under names suggestive of silk are:

Kapok Sun Fast Silks
Maxwell Silk Poplin
Rubberized Raincoats
Lion Brand Sewing Silk
Esskay Unequaled Best Silk
Klostersilk
Subsilk
Sansilk
Silkateen
Japsilk

"Artificial silk" is made from cotton or wood cellulose, and sold under trade name of "rayon silk."

VEGETABLE FIBERS

The fibers of vegetable origin contain only small amounts of protein material and are therefore not so likely to sensitize. However, consideration should be given to the widespread use of these fibers, particularly that of cotton and flax. In addition to these there are other vegetable fibers whose importance in commerce is steadily growing and with which we are coming in contact in increasing frequency. These are being almost entirely neglected from the standpoint of possible sensitization. In this connection, for the purposes of classification, it is interesting to distinguish four groups of fibers, depending on the portion of the plant from which they originate:

1. Seed fibers—cotton, vegetable silk, etc.
2. Stem fibers—flax, hemp, jute, etc.
3. Leaf fibers—New Zealand hemp, Manila hemp, etc.
4. Fruit fibers—coconut fiber, etc.

Cotton.—This is the most important vegetable fiber, both for textile and other purposes. Allergic reactions to cotton have been described and instances have been noted in my practice. Although clinical symptoms usually take place on exposure to cotton in the more or less raw state, such as in the cotton mills, mattresses, etc., still it is not at all unlikely that symptoms of hypersensitiveness may occur on close contact with finished cotton materials.

Some of the uses for unspun cotton are:

Mattress stuffing
Pillows
Stuffing for upholstered furniture
Quilts
Padding for robes, etc.
Medical and surgical purposes

Among the most important fabrics made from cotton are:

Bags	Indian Head
Bird's eye	Khaki
Buckram	Linene
Calico	Marquisette
Canton flannel	Muslin
Canvas	Nainsook
Cheesecloth (cotton)	Net
Chinchilla (cotton)	Plush
Corduroy	Sateen
Cretonne	Ticking
Damask	Turkish toweling
Eiderdown	Velour
Flannel (cotton)	Velveteen
Flannelette	Voile
Gingham	

Cottonseed oil is used for culinary purposes and soap-making chiefly. Some grades of cotton used for stuffing of mattresses and for other stuffing purposes are very poor, containing practically all of the dried seed among the fibers. This undoubtedly acts as an important source of cottonseed hypersensitiveness. It has been called to my attention also that cottonseed is used at times in the manufacture of gin.

It is to be remembered also that there are many varieties and species of cotton-yielding plants. Here, as elsewhere, it is possible that specificity as to species may play a selective part. It is conceivable that one may become hypersensitive to a particular variety of cotton, and the cotton protein used for cutaneous tests may not be the type to which the patient will react. Of course, this is also true of other substances, such as wool, feathers, varieties of dogs, and the like. For this reason, in difficult or puzzling cases, it is sometimes advisable to test the patient with extracts prepared from the particular specimen under suspicion, such as pillow stuffing or rug fibers. In many cases it is a simple matter to test the allergic individual with a composite of all materials in the house capable of giving off small particles. The dust collected from the representative parts of the patient's home constitutes such a mixture and almost always contains the atopens to which the patient reacts.

Flax.—This ranks next to cotton in importance among the vegetable fibers. Flax and its products are occasionally responsible for allergic conditions. Flaxseed is used for poultices and occasionally for other purposes. Linseed oil is used in the making of linoleum, oil cloth, oilsilk, patent and enameled leather, and printers' ink. It is also employed extensively for paints and varnishes and for many brands of furniture polish.[4] Lint is sometimes used for wadding and batting.

The most important linen materials are:

Art linen	Huckaback
Bird's eye (linen)	Sheeting
Cambric	Table linen
Damask	Toweling
Dress linen	Collars and cuffs
Handkerchief linen	Sewing thread

In this connection it is of interest to know that many cotton materials are sold under names suggestive of linen,[5] such as:

French linen
Killarney Linen
Linene
Near linen
Flaxon
Linon

Other Vegetable Fibers.—Jute, obtained chiefly from Bengal, India, is used in large quantities for gunny sacking, burlaps, matting, coat linings, cheapest clothing materials, paper materials, mixed with silk in making cheap satins, velvets, and plushes.

Kapok constitutes the seed hairs from nine varieties of trees belonging to the Bombaceae family. These trees are grown chiefly in the Dutch Indies and in Java. The term "kapok" is sometimes used as a misnomer of other vegetable fibers. Kapok is a soft, light, lustrous, silky material, but the fibers are very fragile and do not lend themselves to the making of textiles. It is used principally in pillows and mattresses, in life-saving belts, and in upholstery. Some recent attempts,[1] partially successful, have been made in spinning fibers from it. "Silk floss" or "floss silk" is a term sometimes used for kapok in bulk.

Bombax cotton is derived from a tropical cotton tree and is principally used for wadding and upholstery material.

There are many varieties of hemp, the most important being Manila hemp. Ropes and cables are the principal products of hemp. Sisal grass is used in large quantities for cordage.

Some of the less important vegetables fibers are: ramie, used for fishing nets and gas mantles; arghan, or pineapple fiber; and coir, or coconut fiber. Vegetable down, the hair fibers of Ochroma lagopus of the West Indies, is used for stuffing mattresses and cushions. Pulu fiber and vegetable silk (Asclepias cotton), so-called milkweed or silkweed, are also used for bedding, pillows, and upholstery. Vegetable wool, obtained by special fermentative process from the green cones of the pine and fir, finds its chief use in mattresses; it is also mixed with cotton and wool in the making of some yarns. "Hygienic flannels," utilized at times for gouty patients, is made from vegetable wool mixed with sheep's wool. Various types of grass-like materials as palmetto, broom-root, rush, palmetto leaves; wheat, rye, barley, and rice straw, and many others are made into brushes, brooms, hats, chair bottoms, floor mattings, sandals and stuffing for mattresses or furniture.

There are several groups of commodities which require somewhat special consideration due either to their importance as possible factors in sensitization or to the general ignorance that prevails concerning their composition. The chief of these are rugs, hats, and furs.

RUGS

Domestic rugs are made chiefly of cotton or wool or a combination of both. For cheap matting, various grass fibers are used. Oriental rugs[6] are

made principally of wool and cotton, but silk, goat hair, camel hair, and linen are used frequently. Oriental rugs differ markedly in their composition, but in general each district produces rugs which are more or less similar in their design as well as in the materials that go into them. It is inadvisable in a presentation of this character to describe all rugs in detail, but the better known and more common types in each class will be considered.

The majority of Persian rugs are composed of cotton and wool, the warp and woof being usually of cotton and the pile of wool. Common examples in this class are the Gorevan, Serapi, Kermanshaw, Ispahan, Saraband, Muskabad, and Kirman rugs. Among the better known rugs containing silk and linen in addition to cotton and wool are the Tabriz and Senna rugs. The Saruks contain cotton, linen, and wool; the Kurdistans are made of pure wool or a combination of wool, camel hair, and goat hair.

The Turkish weavers practically never employ cotton in their rug making. Their carpets are made either of pure wool, or combined with camel hair, goat hair, or silk. The Ghiordes and Mosuls are two prominent types which contain cotton occasionally. Some of the Ghiordes rugs contain silk. In the Mosul rugs goat hair is frequently, and camel hair occasionally, found. The Ladik and Smyrna rugs are of pure wool. The Anatolians are of wool usually, but occasionally goat hair is used for the pile.

Caucasian rugs quite frequently contain goat hair or camel hair or both. Silk is not used among the Caucasian weavers. The Baku weaves contain cotton and wool, and very frequently camel hair is used either for the warp or pile; goat hair is also used but less frequently. The Shirvan rugs constitute a well-known class; these contain goat hair at times. The Kazak rugs are made entirely of wool.

The Turkoman rug makers are very fond of goat hair and use it extensively in their weaving. A good example of this are the Khiva rugs. Several of the Turkoman districts use silk occasionally in their rugs; of these the most prominent are the Samarakands.

Chinese rugs are more or less similar in their composition; the warp and woof are made of either cotton or wool, and the pile is either wool or silk.

HATS

Hats are commonly made of either straw, fur, or felt, wool, cotton, or silk. Straw hats are made of various grasses, including the well-known Panama. Fur caps are not much in vogue at present. But they usually masquerade under false names. From the standpoint of possible sensitization the felt hat is undoubtedly the most important. Hatter's felt is made usually from wool and cotton matted together with other animal hairs. Rabbit hair is used more for this purpose than any of the others. It is not at all improbable that some of the obscure cases of asthma or hypersensitive rhinitis, giving a positive skin test to rabbit hair, owe their trouble to the constant source of exposure to the hair in their hats. Hare, coney, and nutria are also used. Fine felt hats are made from vicuna, camel's hair, beaver, and muskrat; silk is also used in felt hats.

ORRIS ROOT

Orris root is quite a frequent cause of hypersensitiveness, producing asthma, skin disturbances, and hyperesthetic rhinitis. The latter is a particularly frequent result in women. Orris root is the dried, powdered rhizome obtained from certain species of the Iris family—Iris Germanica and Iris Florentina.[9] It is used for its scent, which is that of violets, and for its ability to blend with and hold other scents. The majority of face powders contain orris root, and it is this particular cosmetic that is responsible for the greatest mischief. In addition to face powders it is a common ingredient in perfumes and body powders, and is also found in tooth powder and sometimes in tooth pastes.

No attention is being paid to the possibility of other scents used in cosmetics as a possible cause of sensitization. According to Askinson[10] there are more than 150 natural plant and animal odors used as ingredients in cosmetics. In addition there is a formidable list of synthetic products. The most common animal odors used are: musk, from the muskdeer in the Himalayas; ambergis, from the pot-whale; castor, from the beaver; and civet, from the civet cat and muskrat.

MISCELLANEOUS

There are several groups of substances which will be taken up very briefly at this time.

Hypersensitiveness to glue has been reported in the literature on several occasions. There are two chief varieties of glue: animal glue and fish glue. Animal glue is made[13] by boiling and steaming the hides of horses, cows, sheep, hogs and rabbits, and the bones and cartilages of sheep, calves, dogs, cats, goats, cattle, and horses. Leather wastes are also used. The common variety of fish glue is made from the entire fishes; isinglass is made from the air bladders of the fish. The chief uses for glue are: for joining, as in carpentry work, cabinet making, etc.; binding, as in papier mâché, in mineral colors in manufacturing of colored paper, bookbinding, sand papers, matches; sizing, cloth and paper; isinglass, gelatin and jellies; gelatin capsules, combs, and buttons. Gelatin is also used in the making of ice cream, candies, and commercial preparations for making home products. Gelatin is also used for clarifying liquors,[14] and fish glues are also used for heavy solutions or pastes. Some mucilages and pastes are made from glue; others from gums which exude from the wounded bark of several plants—gum arabic, gum tragacanth, etc. Some pastes contain flour, starch, or dextrin.

Laundry starch, because of its finely divided state, may be a possible factor in causing allergic disease. In this country starch for laundry purposes is made from corn and wheat; in Europe potato starch is chiefly employed. The finest laundry starch is made from rice.

The fats and oils used in soaps are chiefly tallow and grease and cocoanut oil. In addition the following are also used: soya bean oil, cottonseed oil, red oil, palm oil, palm kernel oil, and peanut oil. Various perfumes are used to scent soaps.

Linseed oil is used extensively as a basis for paints. Soya bean oil and China wood oil are also used in large amounts. Turpentine, which is a volatile liquid made by distilling the resin of the pine tree, is employed for thinning paints.

Pyrethrum hypersensitiveness is quite frequently encountered. It is used extensively in insect powders.

Hypersensitiveness to wood has been described on several occasions. This is practically always due to wood in a fine form, such as in sawdust or excelsior. Carpenters, or individuals handling sawdust for cleaning or packing purposes may become sensitized to a particular variety of wood. Excelsior, which is used for packing purposes, stuffing of cheap furniture or mattresses, is a possible source of allergy. Attempts have been made also to use excelsior for other purposes, such as for absorbing material, and for weaving into floor coverings.[11] The excelsior is made chiefly of aspen and yellow poplar pine, but, in various parts of the country, a great variety of woods is used which are native to those districts.

Tobacco as a cause of hypersensitiveness has been observed in several instances. Tobacco is used chiefly for smoking purposes and for snuffing and chewing. Symptoms due to hypersensitiveness to tobacco may occur in nonsmokers as well as in smokers; the presence of tobacco smoke may be sufficient. Two examples of this sort have recently come under my notice.

FURS

The furbearing skins of animals are utilized extensively in this country, and there is hardly a home that has not some article of apparel made of fur. The costly furs are frequently sold under their correct names, but the ordinary varieties are sold under names suggestive of more costly furs. The only furs which the mass of people use are limited to a small group of skins which are practically unknown in the retail market as such.[5] The cheaper furs are clipped, sheared, dyed, and pulled so as to resemble those which are superior in wearing quality and warmth. The National Association of the Fur Industry in its 1925 Year Book[7] describes the situation, outlines the remedies, and includes a dictionary of fur names which is quite extensive. For our purposes it is important to take cognizance of the fact that in considering furs as a possible cause of hypersensitiveness in an individual one must not accept the patient's word as to the kind of furs she possesses. Almost invariably it is something else.

An illustration of the foregoing may be cited. A man, aged thirty, was seen by me in February, 1926, for an asthmatic condition of two years' duration. Protein skin tests showed repeated marked reaction to cat hair. Inquiry revealed that there were no cats at his home at any time. Further inquiry revealed the fact that the patient had a muskrat cap. He volunteered the information, however, that he noticed the asthma particularly in cold weather when he would go outdoors, put on his fur cap and put the laps over his ears. He was requested to take the cap to a reliable furrier for examination, with the result that the "muskrat" cap was discovered to be made of cat fur.

Attention is called to the fact that it is possible to identify the commercial fur hairs microscopically.[8]

The most important furs commonly altered and sold under names of superior kind are:

SPECIES	ALTERED AND SOLD AS:
Hare, dyed	Sable or fox
Hare, white	Fox
Rabbit, white	Ermine
Rabbit, white, dyed	Chinchilla
Rabbit, dyed	Sable
Rabbit, sheared and dyed	Seal, electric seal, Hudson Bay seal, muskrat
Muskrat, dyed	Mink, sable
Muskrat, pulled and dyed	Seal, Hudson Bay seal, electric seal, Red River seal
Mink, dyed	Sable
Marmot (woodchuck)	Mink, sable, skunk
Opossum	Beaver
Goat	Bear, leopard
Fitch, dyed	Sable
Kid, dyed	Lamb
Otter	Sable
Nutria	Seal, beaver, otter

SUMMARY

1. Attention is called to the importance of the relation between allergic disease and household objects.

2. Data are presented showing the many, and generally unsuspected, finished products that are made from substances which are definitely known to cause hypersensitiveness.

3. Attention is directed to the many unsuspected materials and variation in kinds and brands of well-known materials with which we may come in daily contact and which may possibly play a significant rôle in some cases of allergy.

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PREPARATION OF NEUTRAL ACRIFLAVINE SOLUTIONS FOR INTRAVENOUS INJECTION*†

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BURKE and Newton have discussed the factors that must be taken into consideration in the preparation of dye solutions for intravenous injection and in estimating the value of dye therapy in septicemia.[1] The present article describes experiments designed to determine the effect of some of these factors in the preparation of neutral acriflavine solution for intravenous injection.

EXPERIMENTS

Experiment 1.—To determine the P_H of neutral acriflavine solutions when prepared with various solvents.‡

A 1 per cent solution of neutral acriflavine was prepared in tap water, distilled water, 3 per cent sodium bicarbonate solution, Locke's solution, buffered distilled water, and physiologic sodium chloride solution. The buffered distilled water was prepared by adding 0.300 gm. of potassium dihydrogen phosphate and 0.387 gm. of dipotassium hydrogen phosphate to 100 c.c. of distilled water. The P_H of each solvent was determined by a Stiwall comparator, the dye added, the solution filtered and the P_H again determined, within an hour and after an interval of two days, with a potentiometer.§ The results are given in Table I. In all cases the addition of the neutral acriflavine caused the solution to become more acid. Most of the solutions became more alkaline upon standing.

Neutral acriflavine solutions for intravenous use are usually prepared in physiologic sodium chloride solution. The P_H of physiologic sodium chloride solutions varies with the manufacturer of the salt.[2] It also varies with the water used, tap or distilled water, age of the distilled water, and with the application of heat.

It is evident that neutral acriflavine solutions, as prepared and used in practice, vary in P_H. Some patients may receive highly acid solutions and others receive solutions near the neutral point. The effect of a highly acid solution injected intravenously in an animal suffering with infectious acidosis may be serious. Also neutral acriflavine has less bacteriostatic action in an acid medium. Variations in acidity may account for some of the variations in results obtained by different clinicians. However, it probably cannot account for all the unfavorable results obtained in cases treated with acriflavine.

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‡Neutral acriflavine Abbott was used in all our experiments.

§The potentiometer determinations were made by by Dr. J. R. Neller.

As a solvent for neutral acriflavine, in so far as the P_H is concerned, tap water is more desirable than distilled water. Proper buffering will insure a favorable reaction in the solvent and reduce the amount of change occurring as a result of the addition of the dye. A 3 per cent sodium bicarbonate solvent was found to have a P_H of 8.7 and also to be alkaline after the addition of the dye. The application of buffers in the preparation of solutions for intravenous use has been described by Mellon, Slagle, and Acree.[3]

TABLE I

RESULTS OF EXPERIMENT TO DETERMINE THE P_H OF NEUTRAL ACRIFLAVINE SOLUTIONS WHEN PREPARED WITH VARIOUS SOLVENTS

SOLVENT	P_H OF SOLVENT	P_H AFTER ADDITION OF DYE	P_H AFTER STANDING TWO DAYS
Tap water	7.6	7.05	7.73
Distilled water	6.8	6.22	6.29
3% sodium bicarbonate	8.7	8.06	8.59
Locke's solution	7.6	5.71	5.50
Buffered distilled water	6.8	6.37	6.39
Physiologic sodium chloride solution	7.3	6.04	5.34

Experiment 2.—To determine the solubility of neutral acriflavine in various solvents.

One per cent neutral acriflavine solutions were prepared in tap water, distilled water, buffered distilled water, physiologic sodium chloride solution, 3 per cent sodium bicarbonate, 4 per cent dextrose, buffered physiologic sodium chloride with 4 per cent dextrose, and Locke's solution. The solutions, with the exception of the tap water, were prepared with distilled water. After the dye was added the solution was warmed, rotated for thirty minutes, allowed to cool to room temperature, and then filtered through filter paper. The original container, filter paper, and funnel were dried and weighed. The residual matter on them was determined and from this was calculated the amount of dye going into solution. The results are given in Table II, and apparently indicate that the amount of dye going into solution varies with the solvent. This method does not determine accurately the amount of dye going into solution in each solvent. As the dye goes into solution some of the salts precipitate out. With Locke's solution the dried precipitate weighed more than 1 gm., i.e., more than the weight of the dye added. However, some dye remained in solution in the Locke's solvent as indicated by the color and antiseptic power. While this method does not, with the exception of distilled water, indicate the exact amount of dye going into solution it does indicate that a certain amount at least has gone into solution.

Experiment 3.—To determine the stability of neutral acriflavine solutions when prepared with various solvents.

One per cent neutral acriflavine solutions were prepared as in the preceding experiments. After the first few hours the dye solutions were placed in the dark and examined daily for thirty days. The degree of decomposition and precipitation was determined by comparing the staining power on filter paper and the amount of precipitation. A solution was considered

TABLE II

RESULTS OF EXPERIMENT TO DETERMINE THE AMOUNT OF NEUTRAL ACRIFLAVINE GOING INTO SOLUTION IN VARIOUS SOLVENTS

SOLVENT	ESTIMATED PERCENTAGE OF DYE IN SOLUTION
Tap water	0.796
Distilled water	0.9
Buffered distilled water	0.92
Physiologic sodium chloride	0.792
Buffered physiologic sodium chloride	0.794
3% sodium bicarbonate	0.488
4% dextrose	0.404
Buffered physiologic sodium chloride and 4% dextrose	0.68
Locke's solution	?

TABLE III

RESULTS OF EXPERIMENT TO DETERMINE THE STABILITY OF NEUTRAL ACRIFLAVINE SOLUTIONS WHEN PREPARED WITH VARIOUS SOLVENTS

SOLVENT	1	3	5	6	14	30 days
Distilled water	Unaltered				Unaltered	Slight precip.
Tap water			Unaltered	Slight precip.		
Buffered distilled water	Unaltered				Unaltered	Slight precip.
Buffered physiologic sodium chloride	Unaltered			Unaltered	Slight precip.	Heavy precip.
Physiologic sodium chloride	Unaltered			Unaltered	Slight precip.	Heavy precip.
4% dextrose	Unaltered	Slight precip.	Heavy precip.			
3% sodium bicarbonate	Decomposed					
Buffered physiologic sodium chloride with 4% dextrose	Unaltered	Slight precip.	Heavy precip.			

unaltered if the stain on filter paper equalled that of a fresh dye solution and no precipitation could be observed when holding the flask up to the light.

The results are given in Table III. Of the solutions examined the sodium bicarbonate solution was the only one that began to break down within a few minutes after being prepared. When using this solution it is advisable not to allow it to cool and to inject as soon as the dye has gone into solution. All the other dye solutions apparently remained unaltered for hours and some of them remained so for days. It has been the practice to make up a neutral acriflavine solution in physiologic sodium chloride and store it away for future use. Whether such solutions are as satisfactory as fresh solutions is questionable. Our physiologic sodium chloride dye solutions became slightly cloudy, and a fine precipitate formed in from one to two weeks. In our opinion only freshly prepared dye solutions should be used until it has been shown that old solutions are just as satisfactory. The effect of age on the toxicity for body cells and on the bactericidal action is unknown to us. It is possible that dye solutions showing slight precipitation may still be serviceable, but this remains to be determined. Changes in P_H affect the therapeutic value of the dye and these may occur without visible evidence. When necessary to prepare a dye solution a considerable time in advance of its use it should be prepared in neutral distilled water and stored in that form. The

salts can be added just before the dye is to be injected. While the salts tend to cause the dye solutions to become unstable they can be used to advantage if properly applied.

Experiment 4.—To determine the comparative bactericidal action of neutral acriflavine in various solvents.

A fresh 1:100 neutral acriflavine solution was prepared. Measured amounts were placed in flasks. Sufficient amounts of salts were added to make the required solvents. To each flask was then added additional amounts of solvent to make a dye dilution of 1:10,000. The solvents used were distilled water, physiologic sodium chloride, buffered distilled water, buffered physiologic sodium chloride, buffered physiologic sodium chloride with 4 per cent dextrose added, 4 per cent dextrose, 3 per cent sodium bicarbonate, and 3 per cent sodium bicarbonate in physiologic sodium chloride. To 5 c.c. of each of the dye solutions was added 0.1 c.c. of a twenty-four-hour culture of Staphylococcus albus. At intervals up to eighty minutes a loop of the dye organism suspension was plated out by the usual method, two plates being used. Not enough dye reached the second plate to inhibit growth. We expected to find some relation between the amount of dye in solution in the different solvents or the P_H and the bactericidal action of the dye solutions. However, the experiment failed to detect any material difference between the bactericidal action of the freshly prepared dye solutions. In all cases the organism died between the sixty and eighty-minute intervals. Whether comparable results would be obtained in the presence of blood or with older dye solutions remains to be determined. The use of greater dilutions of the dye might bring out differences in bactericidal action. It should be noted in this connection that in general more dye goes into an acid solvent and this tends to counteract the reducing effect of the acid on the bactericidal action of the dye. Furthermore, the method used for determining the amount of dye going into solution does not in all cases indicate the exact amount of dye going into solution.

Various investigators have demonstrated an increase in the bactericidal action of acriflavine with an increase in alkalinity. Eggerth has shown that this increase is slight between P_H 6.2 and P_H 8.[4] The bacteriostatic action, however, increases more rapidly with changes in P_H. The bacteriostatic action is the important factor in the blood stream.

The experiment was repeated with 1:100 and 1:1,000 dye solutions. In no case did the experiments indicate, in so far as the bactericidal action is concerned, a superiority of one solvent over that of the others. The experiment indicates the possibility of combining various substances with the dye without materially affecting the bactericidal action. The advisability of combining with the dye various substances that support the patient should be determined.

Experiment 5.—To determine the comparative toxicity of neutral acriflavine in various solvents.

One per cent neutral acriflavine solutions were freshly prepared for each injection. The dye solutions were prepared in physiologic sodium chloride.

buffered distilled water, and 3 per cent sodium bicarbonate solvents. Injections were made in the marginal ear veins of normal rabbits. The dye solutions were injected at 37.5° C. over a period of ten minutes. Two or more rabbits were used for each injection as occasionally a rabbit will die immediately following a nonlethal dose of dye. The amounts of dye to be injected were calculated each day from the body weights. We used 5 doses rather than determine the minimum lethal dose for each solvent. The rabbits received from 25 mg. to 35 mg. per kilo. Preliminary experiments had indicated that rabbits would live following 4 injections of amounts of neutral acriflavine up to 20 mg. per kilo in distilled water, physiologic sodium chloride, 3 per cent sodium bicarbonate, and 3 per cent sodium bicarbonate in physiologic sodium chloride.

TABLE IV

RESULTS OF EXPERIMENT TO DETERMINE THE COMPARATIVE TOXICITY OF NEUTRAL ACRIFLAVINE IN VARIOUS SOLVENTS

RABBIT	SOLVENT	MG. PER KILO PER INJECTION	DOSES	CHANGES IN WEIGHT				RESULTS
1	Physiologic sodium chloride	25	5	+137	−189	− 68	−290	Died
2	"	25	5	+ 7	−127	− 68	−200	Died
3	"	25	5	+ 13	+ 13	− 30	− 35	Lived
4	"	25	5	+ 10	− 20	− 20	− 60	Lived
5	"	30	3	+ 20	+ 15			Died
6	"	30	4	−205	−360	−200		Died
7	"	30	5	− 10	− 25	+ 10	− 20	Died, 3 days
8	"	30	5	− 30	− 10	− 10	+ 10	Died
9	"	35	5	− 60	−225	−215	− 55	Died
10	"	35	5	+ 45	−175	− 45	− 20	Died, 2 days
11	"	35	5	− 45	− 70	− 45	+ 15	Lived
12	"	35	5	− 30	− 20	− 40	− 75	Died, 2 days
13	3% sodium bicarbonate	25	5	+ 50	− 25	− 25	− 60	Died, 3 days
14	"	25	5	− 30	−120	− 50	−100	Died, 3 days
15	"	30	5	+ 25	+125	+ 10	−285	Died
16	"	30	5	− 30	+ 30	− 25	−220	Died
17	"	35	2	+ 10				Died
18	"	35	3	+ 5	+ 15			Died
19	Buffered dist. water	25	2	+100				Died
20	"	25	5	+ 55	− 25	− 60	−110	Died, 3 days
21	"	30	5	+160	−180	− 50	−130	Lived
22	"	30	5	− 60	− 35	−120	− 20	Lived
23	"	35	5	+105	− 65	− 45	−175	Died, 4 days
24	"	35	5	− 60	−110	−220	− 10	Died

The results are given in Table IV. The experiment fails to demonstrate any appreciable difference in toxicity of neutral acriflavine in the three solvents for normal rabbits. It is the practice to inject neutral acriflavine in physiologic sodium chloride. Such solutions are acid. The other two solvents used in this experiment are more alkaline. Neutral acriflavine solutions prepared with these latter solvents apparently have equal bactericidal action and equal toxicity for normal rabbits to that of solutions prepared with physiologic sodium chloride. We do not know the comparative toxicity for rabbits suffering with infectious acidosis. The more alkaline solutions may be more desirable in such cases. The value of sodium bicarbonate remains to be

demonstrated. While these experiments failed to demonstrate the value of sodium bicarbonate there is much to support the idea that it should be beneficial in the dye therapy of septicemia.

It should be noted that this experiment does not indicate the maximum nonlethal dose or the lethal dose. We used repeated doses rather than single doses. Our rabbits were normal. Animals suffering with septicemia are probably less resistant to the dye. The practice of using not more than 7 to 11 mg. per kilo in infections may be justified. A normal cow gave symptoms of severe shock following one injection of 11 mg. per kilo. The rabbits lost weight following most of the dye injections. All had diarrhea. Rabbits usually lose weight following the injection of much smaller amounts of the dye. Such rabbits ordinarily recover.

This experiment demonstrates that normal rabbits vary greatly in resistance to intravenous injections of neutral acriflavine. An occasional rabbit will live after five daily injections of 30 and 35 mg. per kilo of body weight. Our average rabbits resisted four daily injections of 20 mg. per kilo.

CONCLUSIONS

1. The P_H of neutral acriflavine solutions varies with the solvent and undoubtedly varies greatly as applied clinically. Variations in P_H may account for some of the unfavorable results obtained clinically.

2. The percentage of acriflavine going into solution varies with the solvent.

3. The stability of acriflavine solutions varies with the solvent. Only freshly prepared solutions should be injected intravenously.

4. The dye solvent may be materially modified without greatly affecting the bactericidal action of the dye solution. The possibility of combining with the dye some substance, such as dextrose, that will support the patient without reducing the effect of the dye on the bacteria deserves serious consideration.

5. The best solvent for neutral acriflavine for intravenous therapy is not known. Physiologic saline may be used with acriflavine but not with gentian violet. Tap water, because it is more likely to be alkaline, may be a better solvent than distilled water. We believe a buffered physiologic saline made from an alkaline tap water more desirable than physiologic saline made from distilled water. The buffer salts should be added before the sodium chloride. The dye should be added to the warm solvent. As soon as the dye is dissolved the solution should be filtered and injected without allowing it to cool below body temperature. If ordinary precautions are observed, sterilization by heat is unnecessary. It may be harmful. A solution in which precipitation has occurred should not be used.

6. Normal rabbits vary greatly in ability to resist intravenous injections of neutral acriflavine. Clinical records indicate a similar variation in resistance among human patients. Since normal animals vary it may be assumed that all the variation shown by patients is not due to the type and stage of the infection but that individual variations exist. What is urgently needed

is some method for detecting those particularly susceptible to the dye. Such patients should not be injected or should receive smaller doses than the average patient can resist.

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THE EFFECT OF INTRAVENOUS INJECTIONS OF NEUTRAL ACRIF LAVINE ON THE BACTERIOSTATIC ACTION OF THE BLOOD*†

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IN THE chemotherapy of septicemia it is desirable to know whether the therapeutic agent used is effective as the result of acting on the bacteria or on the host. If it acts as an antiseptic the smaller the number of bacteria in the blood stream the better the chance for beneficial results. Mass action is a factor in the sterilization of the blood stream as well as in the sterilization of other fluids. Ordinarily in the early stages of a progressing infection the number of organisms is smallest and the bacteriostatic agent should be injected as soon as possible. This will be true if the immune bodies play no part. However, if the destruction of the organisms is determined by the combined action of the therapeutic agent and the immune bodies, it does not follow that the earlier the injection the better the chance for cure. The immune bodies are more likely to be present and effective in the later stages of the disease. Furthermore, if the therapeutic agent acts by stimulating the mobilization of immune bodies rather than by acting directly on the bacteria the injection should be made when the mobilization of immune bodies is most likely to take place and be effective. This may not always be in the early stages of the disease.

Raiziss, Severac, and Moetsch[1] found that animals infected with staphylococcus died before the controls when the dyestuff was injected from one to three hours after infection but that beneficial results were obtained if the injection was delayed for forty-eight to seventy-two hours. They suggest that the beneficial effect of the late injection may have been due to the action of the dyestuff on the host, resulting in a mobilization of the immune bodies when these were more effective than in the early stages of the infection.

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A second explanation of the results obtained by Raiziss, Severac, and Moetsch, and one that appears to us to harmonize better with what we know of the action of dyestuffs on the body and on bacteria, is that the dye in tolerated dose has weak antiseptic action on the bacteria. It alone in the early stages of the infection is insufficient to destroy the organisms. But that after the infection has progressed for some time and mobilization of immune bodies has occurred as the result of the infection the combined action of the dye and immune bodies is sufficient to check the infection.

If this is true then we have an explanation of the divergent results obtained both clinically and experimentally. Thus we can state that dye therapy in septicemia appears to be beneficial in those patients who tolerate the dye well if it is injected after the mobilization of immune bodies has begun. If there are no immune bodies the infection may not be materially checked. If there are sufficient immune bodies the patient will recover without the dye. If the patient is particularly susceptible to the dye the injection may be disastrous. Under such circumstances when to inject becomes a serious problem.

The question of whether the dyes in the blood stream act as an antiseptic or on the host is worthy of further discussion and experimentation. The evidence is not altogether conclusive. The clinical evidence, which is evidence obtained without adequate controls, is conflicting as to the value of the dyes intravenously, and how they act on the bacteria. Experimental work on laboratory animals although properly controlled is likewise confusing. The effect of the dyes on the bacteriostatic action of the blood, while attendant with more difficulties than appear on the surface, is more readily and accurately determined than the therapeutic value.

Evidence concerning the effect on the bacteriostatic action of the blood of well-tolerated doses of acriflavine is not favorable. Spencer[2] failed to detect any bactericidal action in rabbit blood following injection of a tolerated dose. Burke and Newton[3] found no increase in the bacteriostatic action of rabbit blood for staphylococcus following the injection of 11 mg. per kilo of body weight. Meleney and Zau[4] detected some bacteriostatic action for streptococcus after the injection of 25 mg. per kilo. No effect was noted following the injection of nonlethal doses of acriflavine. After the injection of 12.5 mg. per kilo the kidney tissue and after 25 mg. per kilo both kidney and liver tissue inhibited streptococcus.

It is obvious that if an antiseptic in amounts tolerated by the average individual increases the bacteriostatic action of the blood, that antiseptic has therapeutic value. There still remains the necessity of determining under what conditions it should be used. If used under any and all conditions of septicemia conflicting results will be obtained. Since the results obtained with neutral acriflavine are conflicting it seems advisable to investigate further the effect of intravenous injections of this dye on the bacteriostatic action of the blood. The following experiments were designed with that object in view.

Experiment 1.—To determine the bacteriostatic action of rabbit blood following the intravenous injection of neutral acriflavine.

One per cent neutral acriflavine solutions were freshly prepared in physiologic sodium chloride, in 3 per cent sodium bicarbonate, and in buffered distilled water. Amounts of each from 15 to 35 mg. per kilo were injected intravenously in rabbits. Ten to fifteen minutes later blood was drawn from the heart. This blood was added, in amounts from 5 to 40 drops, to tubes containing 10 c.c. of 2 per cent agar. The agar was poured into the half of a Petri dish. The other half of the Petri dish contained dye-free blood agar. Strokes of Staphylococcus albus and Bacterium coli were made across both halves of the dish. The results are given in Table I. The experiment was repeated three times with comparable results. There was a very definite bacteriostatic action in the blood of rabbits receiving 35 mg. per kilo of neutral acriflavine. This action was demonstrated for Staphylococcus albus but not for Bacterium coli. Bacterium coli grew on both halves of the dish but Staphylococcus albus grew only on the dye-free blood agar. Five drops of blood from a rabbit receiving 35 mg. per kilo in 10 c.c. of agar inhibited growth of Staphylococcus albus. Forty drops of blood from a rabbit receiving 30 mg. per kilo delayed growth of the organisms for five days but did not entirely inhibit it. Twenty drops delayed growth for three days. The

TABLE I

RESULTS OF EXPERIMENT I TO DETERMINE THE BACTERIOSTATIC ACTION OF RABBIT BLOOD FOLLOWING THE INTRAVENOUS INJECTION OF NEUTRAL ACRIFLAVINE

NO. DROPS OF BLOOD IN AGAR	BACTERIUM COLI MG. PER KG.					STAPHYLOCOCCUS ALBUS MG. PER KG.				
	15	20	25	30	35	15	20	25	30	35
3% Sodium bicarbonate dye solution										
5	+*	+	+	+	+	+	+	+	+	−#
10	+	+	+	+	+	+	+	+	+	−
15	+	+	+	+	+	+	+	+	+	−
20	+	+	+	+	+	+	+	+	+−	−
25	+	+	+	+	+	+	+	+	+	−
30	+	+	+	+	+	+	+	+	+	−
35	+	+	+	+	+	+	+	+	+	−
40	+	+	+	+	+	+	+	+	+−	−
Physiologic sodium chloride dye solution										
5	+	+	+	+	+	+	+	+	+	−
10	+	+	+	+	+	+	+	+	+	−
15	+	+	+	+	+	+	+	+	+	−
20	+	+	+	+	+	+	+	+	+−	−
25	+	+	+	+	+	+	+	+	+	−
30	+	+	+	+	+	+	+	+	+	−
35	+	+	+	+	+	+	+	+	+	−
40	+	+	+	+	+	+	+	+	+−	−
Buffered distilled water dye solution										
5	+	+	+	+	+	+	+	+	+	−
10	+	+	+	+	+	+	+	+	+	−
15	+	+	+	+	+	+	+	+	+	−
20	+	+	+	+	+	+	+	+	+−	−
25	+	+	+	+	+	+	+	+	+	−
30	+	+	+	+	+	+	+	+	+	−
35	+	+	+	+	+	+	+	+	+	−
40	+	+	+	+	+	+	+	+	+−	−

*The plus sign indicates growth.
+− The plus minus sign indicates growth was delayed for several days.
The minus sign indicates no growth.

minimum amount of dye that will increase the bacteriostatic action of the blood remains to be determined. Whether this amount can be tolerated by man is not definitely known. Clinical reports indicate that it can. In this connection it should be noted that the bacteriostatic action of the acriflavine-containing blood was demonstrated in the presence of agar. Agar greatly reduces the action of the dye and also of the blood. Dyes are more effective in a liquid medium. It is difficult to detect a slightly increased bacteriostatic action in acriflavine-containing blood. Since blood is not transparent counting the organisms must be done. The factors of dormancy and increased tolerance of the organisms for the dye interfere with the detection of slight bacteriostasis by counting methods. Burke and Newton tried this method with gentian violet with unsatisfactory results.[3] Stroking the surface of heat-coagulated acriflavine-containing blood is also unsatisfactory as the heat affects the natural antibodies. We tried this method and found that Staphylococcus albus failed to grow on the surface of blood heated to 60° C. for one hour when taken from a rabbit receiving 30 mg. per kilo but grew vigorously on the blood taken from a rabbit receiving 20 mg. per kilo. The organisms were inhibited but not killed. Growth occurred on blood from a control rabbit. Certain factors, such as growth lag and acquired tolerance for the dye, interfere with the determination of slightly increased bacteriostatic action in whole blood. Since we demonstrated increased bacteriostatic action in the blood of rabbits in agar and in heat-coagulated blood following the injection of 30 mg. per kilo, it is safe to assume that the injection of smaller amounts of dye will increase the bacteriostatic action of the blood in the normal rabbit for Staphylococcus aureus. Since this increased action is very temporary, all we can hope for is to shock the bacterial cells sufficiently to enable the body defenses to destroy them. The evidence bearing on whether this actually occurs, or under what conditions it occurs, is conflicting. The dye as applied clinically apparently turns the tide in favor of the patient in some cases and against him in others. Many factors affect the result. These are largely ignored.

The experiment does not demonstrate bactericidal action in the dye-blood agar. The Staphylococcus albus organisms were inhibited but not killed. A loop drawn along the stroke on the dye-blood agar was stroked on fresh agar. Vigorous growth resulted. Five cubic centimeters of blood drawn from the heart of a rabbit ten minutes after the injection of 30 mg. per kilo of neutral acriflavine in buffered distilled water was inoculated with 0.5 c.c. of a suspension of Staphylococcus albus. The mixture was incubated twenty-four hours. Transfer to agar slants resulted in vigorous growth. Apparently the blood of rabbits does not become bactericidal following the injection of that amount of neutral acriflavine.

In the previous experiment tests of the bacteriostatic and bactericidal action were made on blood drawn from the heart within a few minutes after the injection of the dye. The experiment demonstrated bacteriostatic but not bactericidal action. The following two experiments were designed to determine whether there is an increase in bactericidal action in the body of a normal rabbit following the injection of a large dose of neutral acriflavine.

In Experiment 2 the rabbit was killed shortly after the injection of the dye to insure the dye remaining in the blood stream. Dyes are excreted in a few hours. In Experiment 3 the rabbit was not killed for twenty-four hours. In this rabbit the organisms were exposed to the dye in the blood stream for only a short time. Experiment 4 was designed to determine whether the dye had sufficient effect on the cocci to delay the infection and retard death. Sufficient experimental evidence has been presented recently to suggest that the injection of the dye might hasten death.

Experiment 2.—To determine whether Staphylococcus aureus will be destroyed in the body of a rabbit killed immediately after the injection of a large dose of neutral acriflavine.

Two rabbits were injected intravenously with 2 c.c. of a twenty-four-hour suspension of Staphylococcus aureus. Ten minutes later 1 rabbit was killed and placed in the incubator for twenty-four hours. The second rabbit received 35 mg. per kilo of neutral acriflavine in buffered physiologic sodium chloride. Ten minutes later this rabbit was killed and incubated for twenty-four hours. Both rabbits were autopsied and cultures made from kidney and liver were incubated. Luxuriant cultures of Staphylococcus aureus resulted. The experiment failed to demonstrate any bactericidal action in the body of the rabbit receiving the dye. Since the normal defensive mechanism is largely reduced at death it seemed advisable to test for bactericidal action in the living rabbit.

Experiment 3.—To determine whether Staphylococcus aureus will be destroyed in the body of a normal rabbit following the administration of a large dose of neutral acriflavine.

Two rabbits were injected intravenously with 2 c.c. of a suspension of Staphylococcus aureus. Twenty minutes later one of these rabbits received 35 mg. per kilo of neutral acriflavine in buffered distilled water. Twenty-four hours later both rabbits were killed and cultures made from liver, spleen, and kidney. Staphylococcus aureus was recovered in all cases. The experiment failed to demonstrate any bactericidal action in the rabbit receiving the dye. It also failed to demonstrate that the cocci were sufficiently injured by the exposure to the dye during its short period of circulation in the blood stream to enable the natural defensive mechanism to destroy them in twenty-four hours. It should be noted that 2 c.c. of a suspension of a twenty-four-hour culture is a heavy dose. The result might have been different if a smaller dose had been given.

Since our previous experiments had demonstrated an increase in the bacteriostatic action of rabbit blood following the injection of 35 mg. per kilo of neutral acriflavine, it seemed desirable to determine whether this action was sufficient to check a staphylococcus infection.

Experiment 4.—To determine whether the bacteriostatic action of neutral acriflavine in the blood of a rabbit is sufficient to check an infection with Staphylococcus aureus.

Three rabbits were injected intravenously with 2 c.c. of a heavy suspension of a virulent culture of Staphylococcus aureus. Twenty-four hours

later all the rabbits showed severe symptoms and to the same degree. At this time rabbit No. 1 was given 35 mg. per kilo of neutral acriflavine in buffered physiologic sodium chloride. There was marked improvement in two and a half hours after the injection of the dye and in four hours the animal was breathing normally and eating grass. The animal died six days after the injection of the dye. There were typical staphylococcus lesions in kidney, liver, and heart. Rabbit No. 2 was given 4 injections of the same dye solution of 9 mg. per kilo at six-hour intervals. This rabbit received approximately the same amount of dye per kilo as rabbit No. 1 but in multiple doses. The object was to determine whether multiple doses would prove as effective as one large dose. Bacteriostatic action in the blood has never been demonstrated following the injection of 9 mg. per kilo. Approximately this amount has been used clinically. This rabbit did not show the marked improvement of rabbit No. 1 and died three days after the dye injections were begun. Staphylococcus aureus was recovered from the organs. Rabbit No. 3 served as a control. It died within twenty-four hours after rabbits Nos. 1 and 2 began receiving dye injections. Numerous other controls indicated that death was to be expected at this time.

The results obtained indicate that under certain conditions Staphylococcus aureus infections in rabbits can be checked by the intravenous injections of neutral acriflavine. Apparently one large dose is more effective than the same amount of the dye in multiple doses. Our rabbits died, but it should be noted that the progress of the infection was checked even though the dosage of organisms was excessive. It is possible that the animals may have been completely protected from smaller doses. Mass action is an important factor and one frequently overlooked in experimental work. As demonstrated by Kolmer, Schamberg, and Raiziss a certain amount of disinfectant will lead to sterilization of the blood of an animal when inoculated with a restricted number of parasites but will not do so when the number of parasites is greatly increased.[5]

DISCUSSION

The value of neutral acriflavine in septicemia has recently been questioned by Meleney and Zau.[4] They made extensive and well-controlled investigation into the effect of neutral acriflavine on the blood cells, tissues, and on the bacteriostatic action of the blood. They failed to find an increase in the bacteriostatic action of the blood for streptococci following the injection of nonlethal doses of acriflavine. There was bacteriostatic action in some cases following the injection of 25 mg. per kilo. The injection of nonlethal doses in rabbits suffering with hemolytic streptococcus infections hastened death. They conclude that in so far as hemolytic streptococcus infections are concerned neutral acriflavine is not a legitimate intravenous medicament.

Our results differ from those of Meleney and Zau in two important respects. Our normal rabbits withstood much larger amounts of neutral acriflavine. We do not know whether this was due to a different method of preparing the dye solution or to a different make of dye. This suggests

that either the method of preparation of the dye solution or the methods of manufacture are not sufficiently standardized to produce the best results. Some of the conflicting reports can be accounted for on this basis. Secondly, the injection of the dye in our rabbits suffering with an infection did not hasten death but prolonged life. Our rabbits were suffering with a staphylococcus infection and those of Meleney and Zau with a hemolytic streptococcus infection.

It is evident that the experimental work on neutral acriflavine so far described is too limited and conflicting to serve as a basis for adequately estimating the dosage and value of the dye for intravenous therapy. The nature of the conflicting experimental evidence has been presented by Eggerth and need not be repeated here.[6]

Clinical reports indicate that in some hopeless cases the injection of the dye apparently turns the tide in favor of the patient and at other times against him. We should not expect favorable results in all cases. Patients apparently vary in tolerance for the dyes. As our knowledge increases we may be able to select with greater certainty those cases in which intravenous dye therapy is indicated. As Hanzlick has pointed out there is danger associated with all intravenous medication.[7] But in hopeless cases this danger may be a legitimate risk.

All we should hope for or expect in the use of neutral acriflavine in the treatment of septicemia is a slight shock or check to the invading organisms. The dye cannot be used in sufficient strength to kill the organism. The brief exposure to the diluted dye may check them. The body must do the rest. So many factors are operating that we should expect variable and sometimes disastrous results. Some of the factors are subject to control and can be eliminated. It is the function of the experimenter to determine what these are and to eliminate them. A partial list of the variants which affect the results and deserve consideration are: type of organism, number of organisms, variation in virulence, variation in resistance of host to dye and to the organisms, resistance of the organisms to the dye, hydrogen-ion concentration of the solution, variation in the manufacture of the dye, and in the preparation of the dye solution and in its application. The type of infection is important. Success is less certain in those cases in which there are local abscesses discharging fresh showers of organisms into the blood stream. It is thus seen that variations in the host, in the parasite, and in the dye solution deserve consideration before the value of neutral acriflavine as an intravenous antiseptic can be properly determined. Furthermore, we venture the opinion that progress in intravenous therapy will be more rapid when more attention is paid to combining a number of compatible agents, each slightly aiding the patient and reducing shock rather than, as is done at present, depending on the injection of a single agent. The addition of glucose may be beneficial.

The dyes are not perfect blood antiseptics, but they are serviceable and offer the best treatment available under some conditions, and their use is justified until something better replaces them. At present it is impossible to state which of the antiseptic dyes will give the best results under a given set

of conditions. Some of our experiments, to be published, indicate that gentian violet, although more toxic, should in general prove more effective than acriflavine.

CONCLUSIONS

1. The intravenous injection of 30 mg. per kilo of neutral acriflavine in the normal rabbit causes an increase in the bacteriostatic action of the blood for Staphylococcus albus. No increased action for Bacterium coli was demonstrated. The minimum dose that will bring about an increased bacteriostatic action was not determined. The technic used by us failed to indicate an increase in the bacteriostatic action of the blood following the injection of less than 30 mg. per kilo.

2. The intravenous injection of 35 mg. per kilo does not cause the blood to become bactericidal for Staphylococcus aureus.

3. The intravenous injection of an excessive dose of neutral acriflavine does not always hasten the death of a rabbit with a fatal infection of Staphylococcus aureus. It will in some cases delay death.

4. The clinical and experimental evidence as to the value of acriflavine in septicemia is very conflicting. The factors affecting the value of the dye are given in the discussion.

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A STUDY OF ERYTHROCYTE DIAMETERS IN THE NEWBORN*

By Herbert Silvette, Richmond, Virginia

SEVERAL observers[1, 2, 3, 4, 5] have published reports on the mean diameter and the variability of the erythrocytes of the normal adult, as well as the changes noted in the size of the red cells in the various anemias. These reports naturally led to the question as to whether or not the red corpuscles of the normal newborn child differed in size and variability from the figures determined for the normal adult.

For this purpose, only the erythrocytes of normal, healthy babies were measured. The blood was obtained as soon as possible after the time of birth, and in no case after forty-eight hours. The average age of the babies in the

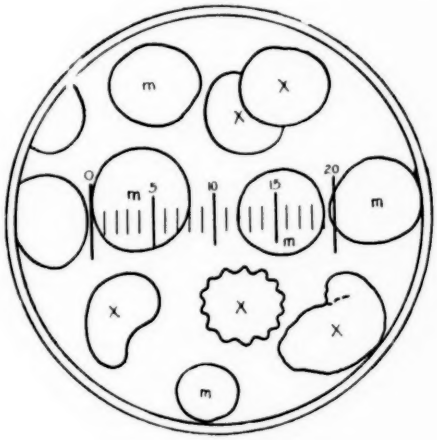

Fig. 1.—Diagram showing micrometer eye-piece in position to measure the red cells.

series was twenty-five hours. The 30 cases in the group represent both males and females; no attention was given the sexes of the subjects in the study.

Blood slides were made by the two-slide method, since it is easily possible in this way to get smears which are thin enough to separate the cells and at the same time avoid the distortion due to too great spreading. Only those cells which retained their natural round or oval shape were measured; those distorted or ruptured by mechanical means, as well as those which were overlapping, were passed over. The diameters of the erythrocytes were measured by means of an oil-immersion objective, together with an 8x micrometer eye-piece, standardized with a Levy-Neubauer counting chamber. Fig. 1 shows

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the method of determining the diameters of some representative cells. The diameters of 200 cells were measured in each case, giving a total of 6,000 cells for the series. The mean diameter of 200 cells measured in each case was taken as the average diameter of the erythrocytes of that particular baby.

The following formula was worked out and used to calculate the mean diameter in each case:

$$\text{Mean Diameter} = \frac{N + N' + N'' + \ldots}{D \times N + D' \times N' + D'' \times N'' + \ldots}$$

where D = a certain diameter, for instance, 5.6 μ.
N = the number of cells of that diameter, i.e., 9.
D′ = another diameter, for instance, 6.4 μ.
N′ = the number of cells of that diameter, i.e., 17.

At the same time that the mean diameters were determined, it was decided to make a red blood count and a hemoglobin estimation on each case.

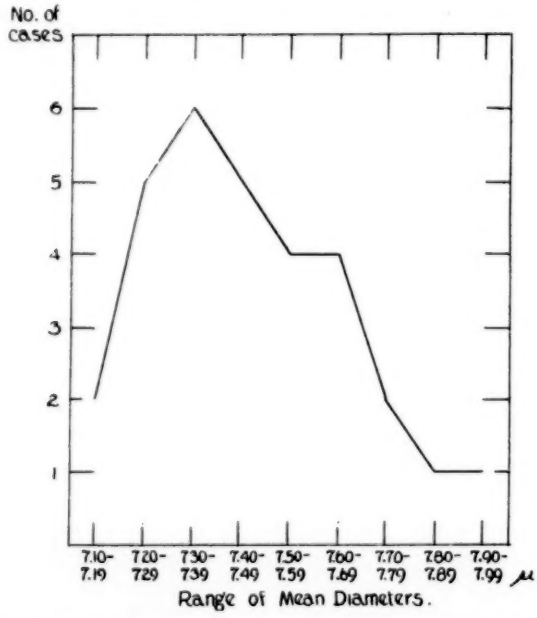

Fig. 2.—Chart showing the distribution of 30 mean diameters between 7.17 and 7.96 micra.

and so to calculate the average color index of the blood of the newborn child. For this purpose, the Newcomer hemoglobinometer, approximately accurate to 1 per cent, was used.

Of the 30 cases, the mean diameters ranged from 7.17 to 7.96 micra, with an average mean diameter for the whole series of 7.45 μ. Fig. 2 is a graph showing the distribution of the mean diameters between the above extremes. The greatest number of mean diameters are massed so as to form a peak very near the average diameter of the whole group. Of the 6,000 cells measured, 17 were 4.0 μ in diameter, 47 were 4.8 μ, 169 were 5.6 μ, 632 were 6.4 μ, 2400 were 7.2 μ, 2391 were 8.0 μ, 261 were 8.8 μ, 74 were 9.6 μ, and 9 were 10.4 μ. The curve plotted using the number of cells measured and their diam-

eters (Fig. 3) is typical in that by far the greatest number of cells form a sharp peak between 7.2 and 8.0 μ, as nearly as it is possible to ascertain with a micrometer ocular. The largest cells measured were 10.4 μ in diameter, while the smallest were 4.0 μ. The difference in size between the largest cell and the smallest in each case—the variability—ranged between 3.2 and 6.4 μ. The average variability of the 30 cases was 4.88 μ.

The following gives, in addition to the figures above, the different blood elements determined along with the mean diameter and the variability of the cells.

Number of babies studied, 30.

Number of erythrocytes measured, 6,000.

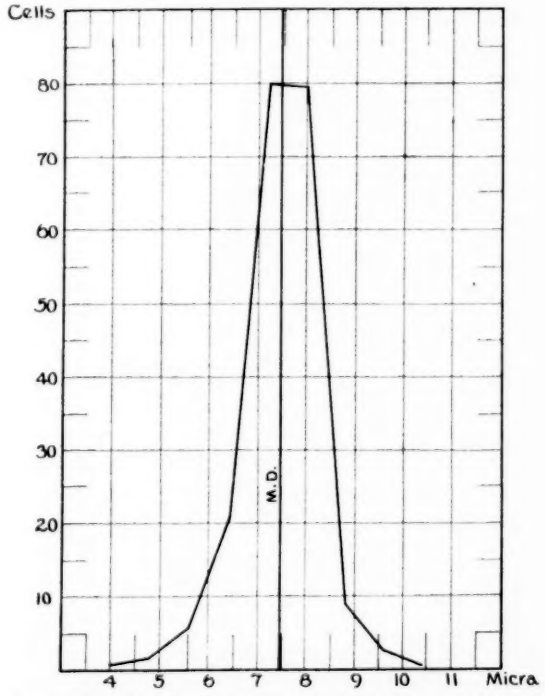

Fig. 3.—Composite chart of red cell diameters of 30 babies; 6000 cells measured; chart reduced to scale of 200 cells; M.D. = mean diameter.

Age of babies, from four to forty-eight hours; average age, twenty-five hours.

Mean diameters, from 7.17 to 7.96 μ; average mean diameter, 7.45 μ.

Variability, from 3.2 to 6.4 μ; average variability, 4.88 μ.

Red blood counts, from 3,280,000 to 5,700,000; average red blood count, 4,519,000.

Hemoglobin, from 71 to 110 per cent; average hemoglobin, 92.9 per cent (average of 20).

Color index, from 0.86 to 1.16; average color index, 1.02.

As the color index is usually high when the size of the cells is increased, as in pernicious anemia, and low when the mean diameter is decreased, as in secondary anemia, it was thought that the two figures would parallel each

other in the case of the babies studied. In a general way this was so in the individual cases, and the averages for the group showed this correlation very well. The mean diameter at 7.45 micra and the color index at 1.02 for normal newborn babies ran parallel to but somewhat higher than the corresponding figures of 7.21[1] μ and 1.00 for the normal adult. In fact, 7.21 : 7.45 = 1.00 : 1.02 is a fairly true proportion.

Fig. 4 is included as a typical chart of the diameters and the variability of the red corpuscles of a normal baby.

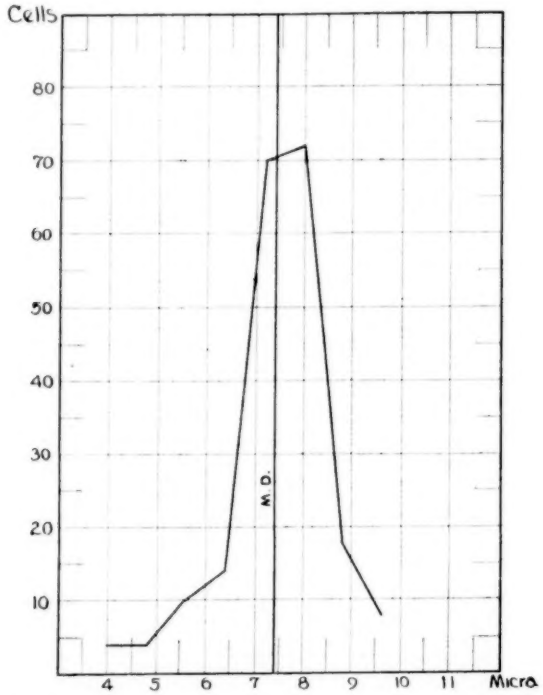

Fig. 4.—Chart of red cell diameters of a typical case. M.D.—7.38 micra. Variability—5.6 micra; R.B.C.—4,250,000; Hb. 86 per cent; 48 hours old. 200 cells measured.

Both the diameters of the erythrocytes themselves and the degree with which they vary in size is very constant, and in these respects the red blood cells of the normal newborn child exhibit the same remarkable constancy as do those of the adult.

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NOTE ON SACCHAROMYCES MALI DUCLAUXI ISOLATED FROM A THROAT CULTURE*

By Rachel E. Hoffstadt, Ph.D., D.Sc., Seattle, Wash.†

THE existence of membranes in the throats of persons, other than those caused by the diphtheria organisms, has frequently been observed. They vary in color, consistency, and persistence. *Oidium albicans* is often found to be the cause of such membranes.

Grover[1] (1916) cultured seventeen varieties from diphtheria cases and diphtheria contacts but named none of them.

Ruth, aged twelve, a healthy child, showing no temperature or other symptoms, developed a persistent white, flaky membrane on the left tonsil. The case was diagnosed from the type of membrane, and on microscopic examination, as *Oidium albicans*. The organism, when cultured on glucose agar, grew abundantly in a moist raised colony, while on agar agar the growth was slow and scant. In broth a sediment was first formed which was followed by the formation of a light, floating scum and "ring" on the side of the tubes. As the cultures aged, the ring, in some instances, reached the top of the tube, giving the tube the appearance of being etched.

It fermented glucose broth with the formation of gas and acid, and glycerin broth, with acid. It did not ferment lactose, sucrose, maltose, inulin, or salicin broths. On potato it formed a moist growth which, after seventy-two hours, extended to the sides of the test tubes opposite the slant. No change was formed in milk. A scanty growth along the line of puncture was seen in gelatin. These cultural reactions agree with the organism two described by Grover.

The yeast cells in young cultures were 6 microns in length and 3 microns in width. They showed little vacuolation when young but became highly vacuolated at the end of forty-eight hours. On solid medium and in the sedimentary deposit of old liquid medium they formed a septate mycelium of from 3 to 11 cells. Each cell was from 1 to 1½ times the length of a parent yeast cell. At this point it might well be mistaken for an *Oidium albicans*.

The cells germinated by budding. After seventy-two hours at 20° C. ascospores are formed in the cells in the scum and on the sides of the glucose broth tubes. During sporulation the cytoplasm becomes highly vacuolated. Regularly 16 basophile granules appeared and were most frequently placed around the edge of the cell. These arranged themselves in groups of eight at the ends of the cell before the formation of the two ascospores. No more than two ascospores were observed. The spores were surrounded by a single

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membrane. No trace of copulation was seen, but the regular arrangement of the basophile granules might indicate rudimentary sexual phenomena. The ascospore germinated in a single direction.

The thermal death point was 55° C. The organism was not pathogenic to guinea pigs.

This yeast agrees in description with that isolated by Kayser[2] from cider, *Saccharomyces mali* Duclauxi. He found it imparted the bouquet to the solution.

Saccharomyces mali Duclauxi resembles *Oidium albicans* in morphology during its yeast-like form, and might be mistaken for it on microscopic examination. It differs from it in that it grows more abundantly on ordinary culture media and ferments glucose with the formation of gas and acid and glycerin, with acid. The type of membrane formed on the throat by *Saccharomyces mali* Duclauxi was similar in appearance to that formed by *Oidium albicans*, but the patient from whom it was isolated showed no temperature or other symptoms.

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LABORATORY METHODS

THE EVALUATION OF BRIGGS' METHOD FOR THE COLORIMETRIC DETERMINATION OF PHOSPHORUS*

By Helen B. Bennett, B.A., New Haven, Conn.

THE colorimetric determination of small amounts of phosphorus according to Bell and Doisy,[1] as modified by Briggs,[2, 3] has been widely and successfully used for the determination of phosphorus in blood. The principle of the method depends upon the formation of phosphomolybdate which is reduced by hydroquinone to give a blue color proportional to the amount of phosphorus present. When this method is applied to physiologic material other than blood, such as food, urine, and feces, the accuracy of the method is still open to investigation. The present study is an attempt to evaluate the optimal conditions of the concentration of reagents, the sulphuric acid concentration, and the salt concentration upon the reduction of phosphomolybdate by hydroquinone, as measured by the blue color developed.

EXPERIMENTAL

The procedure for color development recommended by Briggs in his blood method[3] has been designated the "standard" procedure with which our subsequent determinations have been compared. All the experiments were conducted upon standard solutions of monopotassium phosphate. The usual sodium molybdate, hydroquinone, and sodium sulphite reagents were used. The rate of color production under "standard" conditions is expressed in per cent of that developed in twenty-four hours (Fig. 1). The data show that at thirty minutes the color developed is within 5 per cent of that obtained at the end of an hour and that a five-minute difference in the time of reading causes only a 1 per cent error. At one hour and subsequently, the rate of change is so slow that hourly differences in time do not matter. In from one to five hours 90 per cent of the color value developed in twenty-four hours is obtained.

THE CONCENTRATION OF REAGENTS

The effect of developing the color in a more dilute solution than that specified under the "standard" procedure upon the proportionality of phosphorus has been determined. The limits of the amount of phosphorus which the method can handle have not been adequately defined in the literature. At least 0.2 mg. per cent of phosphorus must be present in order to obtain a color deep enough to read in the colorimeter. The color obtained with a phos-

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phorus content above 2.0 mg. per cent is too deep to read accurately without dilution. The limits of proportionality of the color with the phosphorus content were determined with various final volumes at forty minutes. The amount of reagents were constant throughout. Table I expresses the amount of phosphorus in each solution calculated from the intensity of color relative to that of the others in the same series. Solutions which had a greater phosphorus content than 2.0 mg. per cent were diluted just before being read. This table shows that proportionality is maintained from 0.2 mg. to 3.0 mg. per cent under "standard" conditions. (Column 1.) With two and a half times the "standard" volume, proportionality is kept from 0.2 mg. per cent to 2.0 mg. per cent. (Column 2.) In five times the volume proportionality is maintained from 0.2 mg. to 1.2 mg. per cent (Column 3), while it is maintained from 0.2 mg. to 0.5 mg. per cent in ten times the volume. As proportionality is maintained within these defined limits, it is obvious that the rate

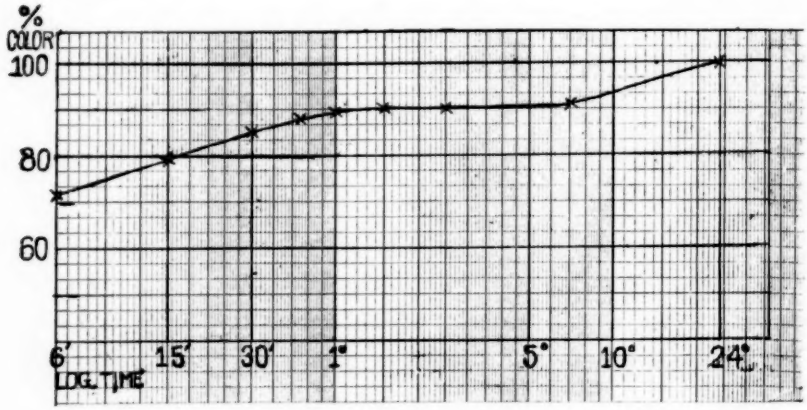

Fig. 1.

of color development with different phosphorus concentrations is the same under the same conditions of reagents. Dilution after the color has been developed does not alter proportionality.

Because proportionality is maintained in the same dilution it cannot be assumed that the same amount of color is obtained with different concentrations of reagents. When the final volume, at the time of the addition of the reagents, is greater than two and a half times the "standard" volume, less color develops. When the reagents are diluted to five and ten times the "standard" concentrations, 85 and 70 per cent respectively, of the "standard" color is obtained as a maximum. This is shown in Fig. 2 where the milligrams per cent of phosphorus present are plotted against the per cent of theoretic color obtained under "standard" conditions, calculated from comparison with 1.0 mg. per cent of phosphorus at forty minutes. Twice the "standard" concentration of the reagents; i.e., one-half the volume does not increase the amount, or the rate obtained up to two hours. When the reagents are present in concentrations three and four times that of the "standard" the color and rate are not increased, but an undesirable greenish tinge is present which

TABLE I

LIMITS OF PROPORTIONALITY OF PHOSPHORUS IN RELATION TO DILUTION OF REAGENTS

10 C.C. VOL. RECOVERED MG. PER CENT	25 C.C. VOL. RECOVERED MG. PER CENT	50 C.C. VOL. RECOVERED MG. PER CENT	100 C.C. VOL. RECOVERED MG. PER CENT	P. CONC. PRESENT MG. PER CENT
0.21	0.20	0.21	0.20	0.20
0.30		0.32	0.31	0.30
0.37	0.39	0.39	0.40*	0.40
0.50		0.51	0.48	0.50
0.58		0.61	0.53	0.60
0.70		0.70	0.52	0.70
0.78	0.80*	0.80*	0.51	0.80
0.93				0.90
1.00		1.00		1.00
1.20	1.20	1.20		1.20
1.40		1.20		1.40
1.60*	1.60	1.20		1.60
1.80		1.20		1.80
2.0	1.98	1.20		2.00
	2.00			2.20
2.30				2.40
				2.60
2.70				2.80
2.90				3.00
2.70				3.20
				3.40
				3.60
2.50				3.80
				4.00

*Standard.

interferes with comparison. Hence, the concentration of reagents should be the same in the standard and in the unknown, and for optimal conditions should approach the "standard" concentration. This error introduced by variations of conditions has been recognized by Bell and Doisy,[1] Stanford and Wheatley,[4] and by Fiske and Subbarow.[5]

The effect of changing the concentration of each reagent upon the color development was determined when the other reagents and the final acidity were maintained under "standard" conditions. It was found that the limits of the reagents at a final acid concentration of 0.25 μ are as follows: molybdate, 0.5 to 1.0 per cent (10 to 20 c.c. of 5 per cent ammonium molybdate solution); hydroquinone, 0.05 to 0.25 per cent or more (5 to 10 c.c. of 0.5 per cent hydroquinone in 100 c.c.); sulphite, 0.2 to 2.0 per cent (1 to 10 c.c. of 20 per cent sodium sulphite solution in 100 c.c.). In agreement with Roe, Irish, and Boyd[6] it was found that the molybdate reagent itself is reduced above the given limit. Excess sulphite prevents reduction of the phosphomolybdate.

THE SULPHURIC ACID CONCENTRATION

The effect of acid upon the color production was determined when the concentration of reagents was kept as defined under "standard" conditions. The results obtained when the final acidity was varied from 0.05 μ to 1.0 μ are shown in Fig. 3. The maximum stable color is observed in acid concentrations of 0.05 μ to 0.50 μ in from one to three hours. A final acid concentration of 0.25 μ, which gives a stable color in from fifteen minutes to

twenty-four hours and which is the midpoint of this acid range, is, therefore, a safe acid concentration to use for a standard. This agrees with the conclusion of Stanford and Wheatley[4] and Martland and Robison.[7]

THE SALT CONCENTRATION

The influence of salt upon color development is dependent upon the acidity, the concentration of reagents, and their proportion to each other in the final volume of the mixture. An investigation into the question could be indefinitely broad in its scope. An attempt has been made to determine the effect of some of the common salts which are formed on neutralizing the ash of physiologic material.

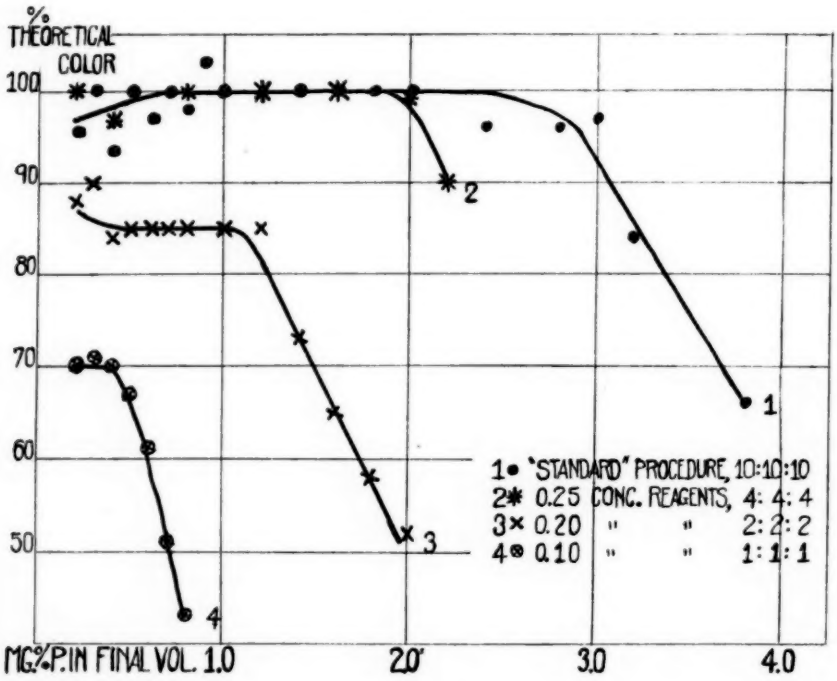

Fig. 2.

The effect of salts upon the amount of color developed has been determined under "standard" conditions. These solutions were compared to a similar salt-free standard made up at the same time. The molarity of the salts has been defined in terms of their concentration in the colored solution. The salts studied in concentrations of from 0.2 to 1.0 m were sodium chloride, potassium chloride, sodium nitrate, ammonium nitrate, magnesium nitrate and sodium sulphate. The salt effects are small. The concentration where the salt effect is first noted differs for the various salts studied. The mono-mono salts (sodium chloride, potassium chloride, sodium nitrate, and ammonium nitrate) do not influence the color up to 0.6 m at thirty minutes. Sodium sulphate effects the color at 0.3 m and magnesium nitrate at 0.2 m. Several writers have recognized that salts

interfere with the color production.[3, 5, 8] In general, the effect is to increase the color at first and later to diminish it. For practical purposes it can be said that when salts of unknown types and amounts are present it is best to compare the colors for from forty-five minutes to one hour. All the errors will not be avoided in this way, but when the reagents are present in proportions and amounts described under the "standard" procedure, the probable error may be assumed to be plus or minus 5 per cent.

When the solutions are diluted five times the "standard," no augmentation of the color takes place during the early stages of color development. There is a general retarding effect in some cases, amounting to 70 per cent in fifteen minutes, which is marked even at low salt concentrations. This does not affect the amount of color developed later for in nearly all cases normal values are obtained in three hours. Hence, dilution of reagents should be avoided.

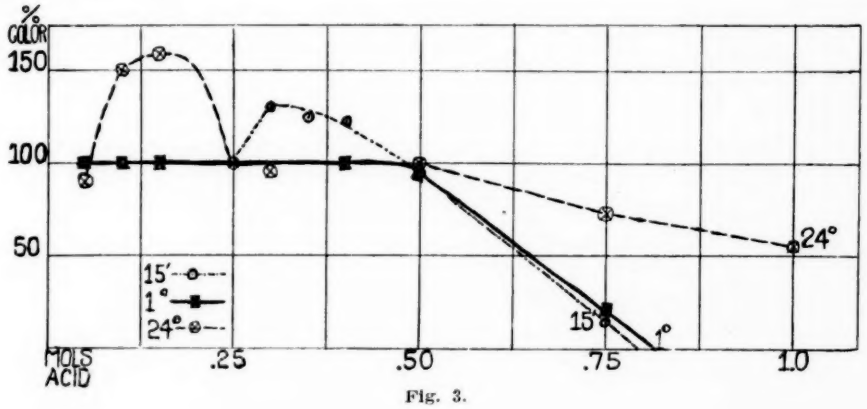

Fig. 3.

In order to ascertain the magnitude of error introduced by a combination of salts, a comparison between the usual gravimetric and colorimetric determinations was made upon urine and stools. The errors are of the same order of magnitude as those obtained with individual salts. The phosphorus determined in unashed urine with five times the volume of the "standard" at one hour gave errors from −35 per cent to +66 per cent. In rat stools of low phosphorus content, when the reagents are diluted to two and a half times the "standard" or in the "standard" volume, an average error of plus or minus 5 per cent is obtained.

The effect of salt concentrations when the acid is also present in varying amounts was determined in the case of sodium sulphate under "standard" conditions. The solutions were compared with the salt-free standard of the same acidity, developed simultaneously. In the optimal acid range (0.05 m to 0.5 m) 0.01 m to 0.2 m sodium sulphate does not interfere. Greater salt concentration (up to 1.0 m) causes an unstable color and depending upon the conditions either augments or decreases the rate of color development. A 35 to 160 per cent increase in the color intensity is obtained with all concentrations of sodium sulphate used (0.01 to 1.0 m) in 0.75 m acid at all times.

ADOPTED TECHNIC

The above results show that the conditions defined by Briggs for the determination of phosphorus in blood are correct. However, it is safer to change the amounts of the reagents to 15 c.c. of molybdate containing 1.6 ɱ sulphuric acid, 15 c.c. of hydroquinone, and 5 c.c. of sulphite per 100 c.c. final volume; or, keeping the convenient volume relationship of Briggs, to use the following reagents:

TABLE II

A COMPARISON BETWEEN GRAVIMETRIC AND COLORIMETRIC PHOSPHORUS DETERMINATION OF STOOL ASH

	HIGH P BRIGGS NEW					LOW P BRIGGS NEW				
	*GRAV. MG. PER CENT	AUTHOR'S REAGENTS		BRIGGS' REAGENTS		*GRAV. MG. PER CENT	AUTHOR'S REAGENTS		BRIGGS' REAGENTS	
		MG. PER CENT	ERROR PER CENT	MG. PER CENT	ERROR PER CENT		MG. PER CENT	ERROR PER CENT	MG. PER CENT	ERROR PER CENT
1 B	1.94	2.03	+ 5	2.0	+3	0.39	0.37	–5	0.37	–5
2 B	1.80	1.98	+10	1.89	+5	0.36	0.36	0	0.36	0
3 B	1.90	2.0	+ 5	1.92	+1	0.38	0.36	–5	0.37	–3
4 B	2.04	2.06	+ 1	2.0	–2	0.41	0.38	–7	0.38	–7
5 B	2.2			2.11	–4	0.44	0.41	–7	0.42	–4

*The amount of phosphorus present in the aliquot taken for the colorimetric determination is calculated from the gravimetric value.

Acid Molybdate Reagent.—

37.5 gm. of ammonium molybdate are dissolved in 300 c.c. of water and to this are added 200 c.c. of water containing 75 c.c. of concentrated C. P. sulphuric acid.

Hydroquinone Solution.—

0.75 gm. of hydroquinone is dissolved in distilled water and diluted to 100 c.c. A drop of concentrated sulphuric acid is added as a preservative.

Sulphite Solution.—

A 10 per cent solution of sodium sulphite.

When salts are present, the solution to be analyzed should be diluted if possible. An amount of phosphorus of 0.3 to 0.5 mg. should be used and the color developed in 100 c.c., using 10 c.c. of each of Briggs' reagents (or those given above). In this way, the proportionality of phosphorus is maintained, and the salt effect is diminished to a minimum. The colors should be compared at the end of an hour.

APPLICATION TO STOOL ASH

This technic, which was developed for pure salts, was tested on physiologic material. The solution of the ash of low phosphorus rat stools was determined gravimetrically as magnesium pyrophosphate, by Briggs' blood technic and by the new technic. The results given in Table II show fair agreement between the gravimetric and colorimetric methods. With small amounts of phosphorus negative errors up to 7 per cent resulted with both

colorimetric procedures. With a larger amount of phosphorus, positive errors (1 to 10 per cent) resulted with Briggs' reagents, but correct values (4 per cent) were obtained with the new reagents.

SUMMARY

1. Briggs' method for the determination of phosphorus has been studied in respect to the following factors:

a. The rate of color development.
b. The limits of phosphorus content.
c. The limits of concentrations of reagents.
d. The salt effects at various concentrations, singly and combined and in the presence of various acid concentrations.

2. Based on this study, Briggs' reagents have been found to give correct but not optimal conditions, and alternative reagents have been recommended.

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A NEW AND IMPROVED INJECTION APPARATUS*

By Louis Gross, M.D., New York City

THE study of vascular architecture and vascular disease processes by means of injection has assumed so much importance of late that it was thought advisable to describe an improved apparatus which simplifies and standardizes the technic to a point where it is practically foolproof.

The importance of a standardized uniform injection technic will be appreciated when it is realized that the old methods of injecting vessels by hand, using a syringe, can give no reliable comparative results inasmuch as it is impossible in this way to determine whether differences in the vascular tree are due to artefact or were present in the vessel structure itself.

Elsewhere, I described an injection cabinet which gave excellent results and permitted of fairly accurate standardization of all the technical factors concerned in injection of vascular channels.[1] This cabinet, however, demanded a certain amount of personal control and observation. In order to reduce the personal factor to a minimal degree, the present apparatus was devised. By its use, extraordinarily beautiful and complete injections can be obtained and, since every step in the injection is automatically controlled and standardized, there is left very little room for error or personal equation.

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The apparatus as a whole can be divided into three sections:

1. Pressure section.
2. Saline section.
3. Injection section.

1. PRESSURE SECTION

The pressure section (Fig. 1) is a unit which delivers gas (usually air or oxygen) at a constant pressure. It is alternately attached to the saline section (Fig. 2), in order to wash out the vessels with a stream of fluid under constant temperature and pressure, and to the injection section (Fig. 3), in order to inject the vessels at a constant temperature pressure, and humidity.

It consists of a tank (*A*) containing compressed air or oxygen, to which there is attached a Hoke Phoenix gas pressure regulator (*B*). This regulator has a dial (*C*) which shows the amount of gas in the tank, and another dial

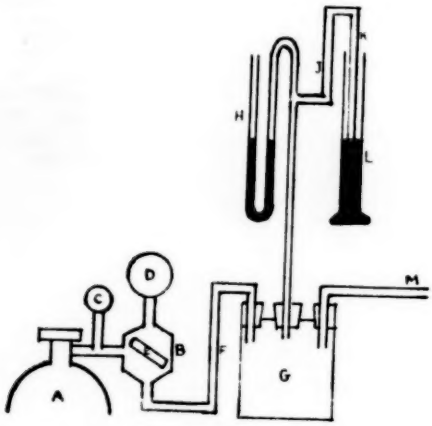

Fig. 1.

(*D*) which shows the pressure of the escaping gas. The latter is regulated by the handle (*E*). The outflowing gas is conducted through pressure tubing (*F*) to a one liter Wolff bottle (*G*) having three necks with perforated rubber stoppers. Into one stopper there is inserted the pressure tube (*F*). The center stopper is connected to a mercury manometer (*H*) which shows the pressure at which the injection fluid is entering the vessels. A rubber tube (*J*) leading from one limb of the manometer is connected to a glass tube (*K*). This is inserted into a column of mercury contained in a cylinder (*L*). By raising or lowering the tube (*K*), and allowing sufficient gas to escape from the tank (*A*) so that a small amount bubbles through the mercury in the cylinder (*L*), an automatic means of controlling the pressure in the system is obtained. The third stopper carries *pressure tube M*. It is this tube which is alternately attached to the saline and injection systems.

2. SALINE SECTION

The saline section (Fig. 2) comprises a unit for automatically delivering saline (or other washing fluid) at a desired constant temperature.

The *pressure tube* (*M*) is attached to the inflow tube (*N*) of the demijohn (*O*). The latter contains normal saline or other fluid which it is desired to wash through the vessels. This fluid is thus forced through the outflow tube (*P*) and through the block tin spiral (*Q*). The latter consists of forty feet of block tin tubing, ⅛ inch internal diameter. The tubing is bent into a long spiral and is inserted in an agate ware (or other metal) pot (*R*). The outflow end of this block tin spiral (*Q*), is led into a small bottle (*S*). Here the washing fluid flows past the bulb of a thermometer (*T*) where its actual temperature just before entering the organ is indicated. For the sake of simplicity, the bottle (*S*) is shown in Fig. 2. In practice, however, it is inserted into the injection cabinet (Fig. 3) in such a manner that the thermometer (*T*) projects through the roof of the cabinet.

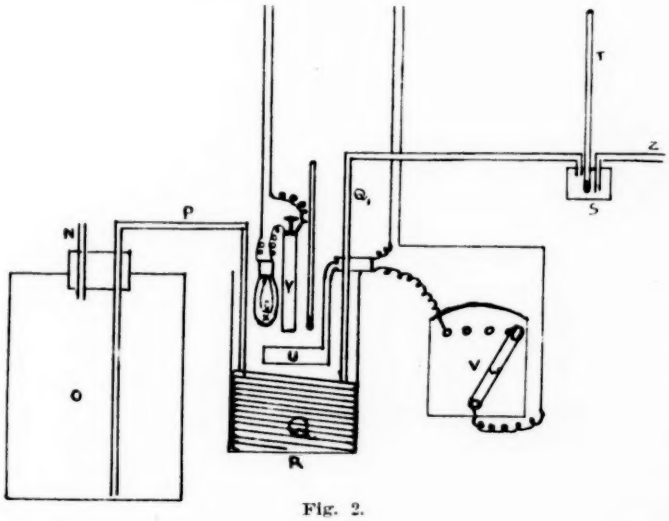

Fig. 2.

The agate ware pot (*R*) is filled with water. The latter is heated by an immersion electric heater (*U*) which is controlled by a rheostat (*V*). In order to bring the water quickly to the desired temperature, the rheostat is so regulated that little or no resistance is offered to the current. After the desired temperature is obtained, resistance is thrown into the current by moving the handle (*W*) to a point on the rheostat where, by previous trial, it is found that the temperature of the water in the pot (*R*) will go no higher than four or five degrees below the desired temperature. Thus, if it is desired that the washing fluid shall be 47° C. the handle (*W*) of the rheostat is moved to a point where the resistance will not allow the heater (*U*) to keep the water at a higher temperature than approximately 43° C. This point on the rheostat is marked. The fine regulation to the exact degree desired is obtained by the use of a 35 or 50 watt carbon filament lamp (*X*) which is attached in series with a bimetallic thermoregulator (*Y*). This type of electric thermoregulator can handle the small current carried by the carbon lamp and is quite sufficient to supply enough additional heat to the pot (*R*) to keep the temperature exactly at the required point.

Obviously the temperature of the pot (*R*) must be maintained at such a point as will deliver a current of washing fluid from (*Z*) at the desired temperature (usually 45° C.). As indicated before, the fluid is conducted from the saline section into the injection section, through the bottle (*S*).

3. INJECTION SECTION

The injection section (Fig. 3) consists of a wooden cabinet having a glass window (*1*) as a roof, and two doors which open in front. One door is large, the other small; the division between the doors being opposite the copper partition (*2*).

The entire cabinet is lined with copper, and is divided by a dwarf partition (*2*) into a smaller (*3*) and a larger chamber (*4*). The smaller chamber holds the rocker (*5*) of a shaking machine. The rocker is attached by means

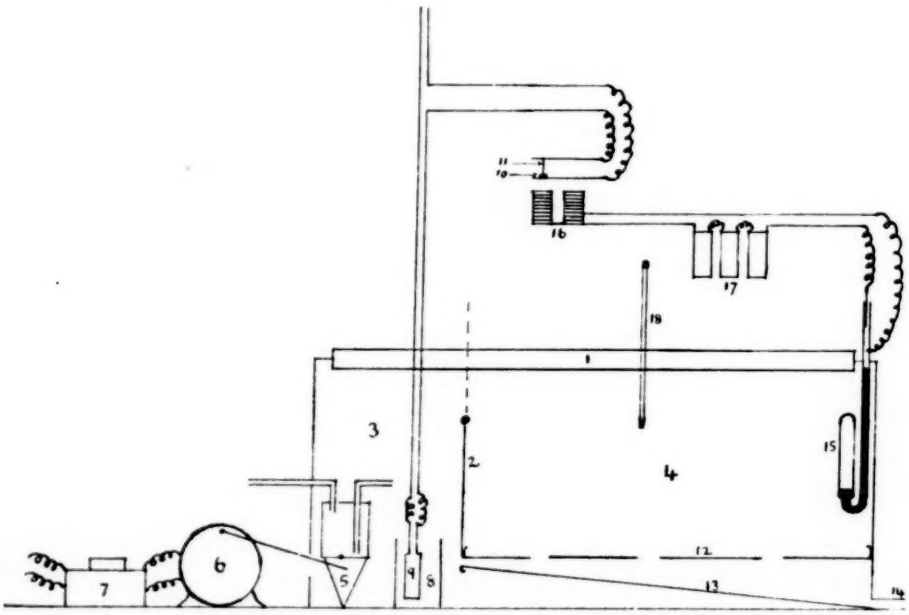

Fig. 3.

of a shaft passed through a slit in the cabinet, to the eccentric wheel of the shaking machine motor (*6*). The speed of the rocking is controlled by a rheostat (*7*).

The smaller chamber (or larger chamber) contains a pot (*8*) holding water in which there is placed an immersion electric heater (*9*). The circuit of this heater is closed by the contact of a disc (*10*) (belonging to an electromagnetic relay), with a platinum pin in the circuit (*11*).

The floor of the larger chamber consists of a perforated and removable copper pan (*12*). Beneath this there is a sloping floor (*13*) which conducts waste fluids through the drain pipe (*14*).

Through the roof of this chamber there is inserted a toluol-mercury thermoregulator (*15*) which opens and closes (by means of the mercury columns) a circuit energizing the electromagnet (*16*). Several dry batteries

(*17*) or other sources of current, are placed in series through this circuit. A thermometer (*18*) records the temperature of the large chamber.

By this method a very accurately and automatically controlled temperature is obtained in the large chamber. The immersion heater (*9*) evaporates the water in the pot (*8*) and raises the temperature in the cabinet. The excess moisture condenses and the humidity is therefore practically constant for a given temperature. As soon as the temperature reaches the desired level, the mercury columns in (*15*) close the circuit energizing the electromagnet (16). The latter attracts the disc (*10*) and pulls it away from contact (*11*). This in turn breaks the circuit and cuts off the current from the heater (*9*). The temperature then begins to fall, the mercury column in (*15*) contracts, the electromagnet circuit is broken, the disc (*10*) springs back to contact (*11*), and the heater is immediately put on again. It is advisable to put a drop of cedar oil between contacts (*10*) and (*11*) to avoid sparking.

In review, we have three sections: the pressure section automatically delivers gas at constant pressure; the saline section automatically delivers washing fluid at a constant temperature; the injection section keeps the organ to

Fig. 4.

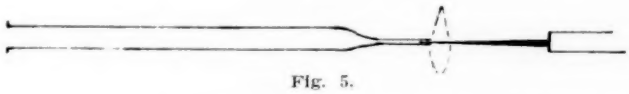

Fig. 5.

be injected in a cabinet at constant temperature and humidity. Morever, the fluid to be injected is agitated by a standardized motion so that if a suspension of particulate matter is used, such as a barium sulphate suspension, a uniform injecting medium is obtained.

TECHNIC OF INJECTION

A typical example of injection to serve as an illustration will suffice to explain the technic employed. If it is desired to inject the coronary arteries of the heart, glass cannulas are inserted into the orifices of the coronary arteries, and are tied into these vessels by means of silk thread. Since the first branches from the coronary arteries are often given off very close to their origin, it is necessary to employ cannulas having a very flat flange. The cannulas are best made in the laboratory and by means of the following technic, they can be made strong enough and at the same time fine enough to be inserted into the coronary arteries of the hearts of newborn infants.

Pieces of glass tubing, 3 mm. internal diameter, 5 mm. external diameter and about 15 cm. long are used. The center of the piece of tubing is softened in the flame of a blow pipe, slightly thickened and drawn out (Fig. 4). The drawn-out ends are nicked with a file about 1 cm. from the slope of the shoulder, and broken off.

The nozzle is dipped into talcum powder, a teasing needle is also dipped into talcum powder, and its point is inserted into the lumen of the nozzle (Fig. 5).

The tube is slowly rotated around the point of the needle and is gradually introduced into the flame of a blow pipe. It is necessary that the flame be kept small. The tip of the

nozzle immediately begins to thicken and the lumen is kept open by the needle. The purpose of the talcum powder is to prevent the needle from fusing with the glass. When the desired thickness of flange is obtained, the tip is removed from the flame, and the opposite end of the tube is rounded in the flame.

The heart is suspended on a tripod, by means of a glass rod which is inserted beneath the pericardial bridge connecting the great vessels, and is placed in the larger chamber. Warm saline (45° C.) is forced through the coronary arteries until the washings are free of blood (about fifteen to twenty minutes). The pressure at which the washing is carried on should be about 180 mm. Hg.

The pressure tube is now applied to the inlet tube of a Wolff bottle which is mounted on the rocker and which contains the injecting medium.[1] If the injecting medium consists of a suspension, such as barium sulphate in gelatin, the rocker is set in motion so as to keep the suspension uniform. The injecting medium is forced through the outflow tube of the Wolff bottle, through the cannulas into the coronary arteries. The pressure is again maintained constant.

When the arteries are completely injected, as can be judged from the fact that after shutting off gas, the mercury column in the manometer does not fall (this takes from twenty-five to thirty minutes), two clamps are placed on each rubber tube connected to the cannulas. The rubber tubing is cut between the clamps. The heart is immediately plunged into cold water, excess barium is washed out of the heart chambers by a stream of cold water, or by means of suction, and the heart is immersed in 10 per cent formalin. After twenty-four hours the heart may be opened and such studies made on it as desired. If it is desired to render the heart transparent, the Spalteholtz method or a modification[1] may be used.

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AN OPERATING BOARD FOR RABBITS*

By Emil Weiss, M.D., Chicago, Ill.

SEVERAL apparatuses for the operative work on rabbits have been described and recommended. A good apparatus of this kind should fulfill two conditions: First, the animal should be securely fixed without injury, and secondly, the position of the animal should be such that it will not impede the intended operation. Usually two different positions are required: First, the fixation on the back, and secondly, on the abdomen. For operations on the abdomen, thorax, and neck the dorsal fixation is required, and for operations on the head the abdominal fixation is preferable. The majority of apparatuses are useful for either one or the other position, but not for both.

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We have devised an apparatus, which has some advantages over the present ones in use. The apparatus is attached to a revolving chair, and to the feet of the chair are affixed castors. By this arrangement the apparatus is portable, rotatable in horizontal direction and can be raised at convenience. The stability of the apparatus is assured by placing a twenty-five-pound weight on the bottom of the chair. The apparatus itself is composed as follows: A horizontal board is screwed to the chair in place of the seat-board. To one end of the horizontal board is attached a shorter vertical board; this board is connected on the upper end by hinges with another board, which can

Fig. 1.

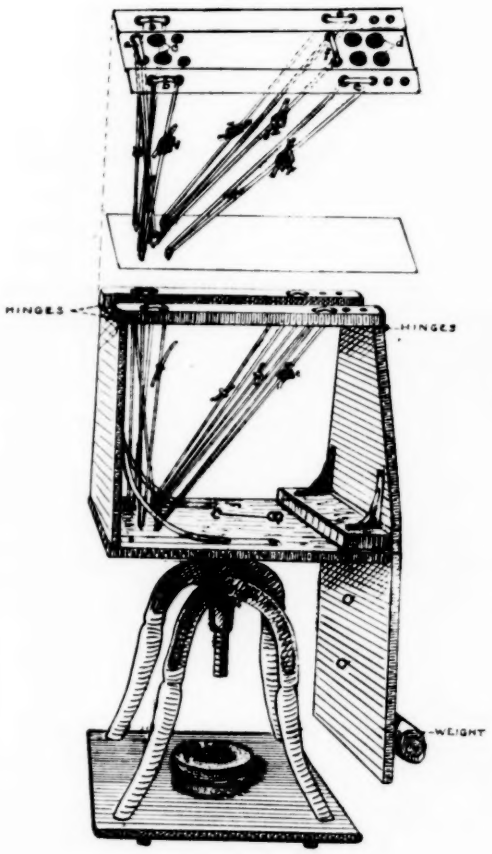

Fig. 2.

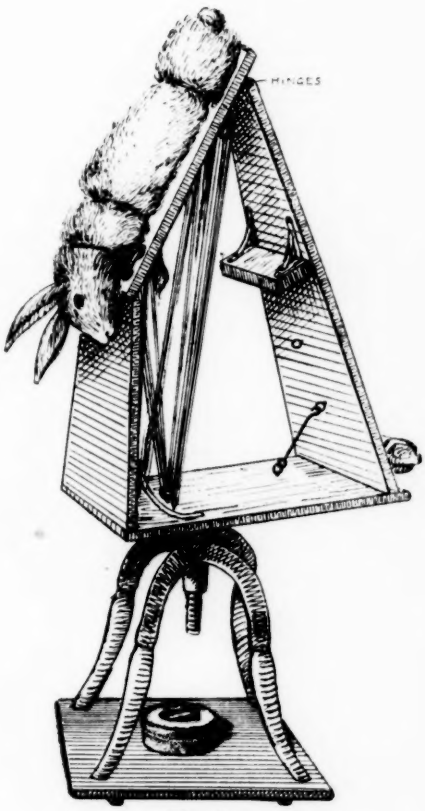

Fig. 3.

be raised from horizontal to vertical position. The last mentioned board contains larger and smaller holes. The large holes are intended for the feet of the rabbits, the small ones for the rubber tubings, used for the fixation of the animal. This board and the horizontal board attached to the chair are of the same length. The perforated board is connected on the other side by hinges with a board twice its length. This board is resting on the lower horizontal board by means of a small piece of wood, which is screwed on to the inner side of the long board; in this position of the long board the horizontal

and the perforated boards lie parallel. To the lower end of the long board is attached a weight of five pounds. The long board can be raised in vertical resp. oblique direction, which causes a simultaneous raising of the perforated board from its horizontal position to a more or less oblique position. This particular position of the fixed animal is especially desirable for intraperitoneal injections with head low and abdomen high, which causes the fall of intestines toward the diaphragm and avoids injury to the intestines by the intraperitoneal injections. The same position is also desirable for injections in the auricular veins, which become more and more dilated and greatly facilitate these inoculations. To a certain extent heart punctures are more easily made in this position of the animal, due to the fact that the beating of the heart is accentuated.

The animal is fixed to the board, the abdomen downward, by pulling the fore feet through holes *c*, and the hind feet through holes *d*. The back of the animal is fixed by a rubber tubing going through holes *f;* the neck of the animal is fixed by a rubber tubing going through holes *a*. A position for abdominal exposure is obtained by pulling the animal, which is lying on its back, through rubber tubing *f*, and fixing the extremities with rubber tubings *b* and *e*. Each tubing from the perforated board goes through the respective eye-screw, which is fastened to the lower horizontal board. The ends of the rubber tubings are held fast by a Hoffmann clamp, which affords an adjustment in either shortening or lengthening of the tubing.

Fig. 1 shows the arrangement of the holes and of the tubing on the perforated board and the fixation of the tubing on the lower horizontal board. Fig. 2 shows the entire apparatus and its attachment to the swivel chair. Fig. 3 shows the raised perforated board with a rabbit in a fixed position.

This apparatus insures a satisfactory fixation of rabbits in various positions without any injury; the fixation of the animal can be performed easily by one person. It can also be used for operations of any kind on cats. We have been using this apparatus for several years in the medical school and hospital laboratories.

NOTES ON BASAL METABOLISM*
XI. BASAL METABOLISM STANDARDS

By William H. Stoner, A.M., M.D., Philadelphia, Pa.

INTRODUCTION

THE purposes here are first to present in outline the several standards of comparison of basal metabolic rate in use at present, second to compare the results obtained in the use of these several standards, third to recommend a choice of standards, and fourth to evaluate the importance of such a choice. The older aspects of the historical development of calorimetric standards are not presented. Only the present status of the subject is outlined.

The various normals of comparison of basal metabolic rate in use at present are very unsatisfactory. This is true of the standards for adults, and those for children are even more unsatisfactory. Several wholly different methods of comparison for adults and for children are in use. Each method gives a different result from the same experimental data. The magnitudes of the variations between these several results are the subject of much difference of opinion expressed in the literature. Each calorimetrist is convinced that the particular set of standards he uses is superior to all the others, yet his knowledge of the advantages and disadvantages of each and of the variations between them is, as a rule, quite uncertain.

STANDARDS

Aub and DuBois Standards.[1]—The oldest of the several systems of standards now in use are those published from the Sage Institute. They consist of a tabulation, for males and females between ages fourteen and eighty years, by two-year periods from fourteen to twenty years and by decades from twenty to eighty years, of the normal number of calories per hour per square meter body surface based on the DuBois and DuBois height-weight formula.[2]

*From the Biochemistry Laboratory and the Department of Metabolic Diseases of the Graduate School of Medicine of the University of Pennsylvania. Read before the American Association for the Study of Goiter, Feb. 1, 1926, at Louisville, Ky.

Received for publication, March 15, 1927.

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IV. Selection of Normal Standards, Boston Med. and Surg. Jour., 1923, clxxxix, 236.
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Although a smooth curve of these values for males accompanied the original communication, the uninterpolated step curve values have been used generally. The values for females are approximately 7 per cent below those for males.

The most frequent criticism of the Aub and DuBois standards, as they are generally used, is that they are not interpolated between the decades above twenty years, nor between the two-year periods below twenty years. For example, the normal heat production by these standards of a man thirty-nine years old will be the same as that of a man twenty years old and of the same body surface, and 2.5 per cent more than that of a man forty-one years old and of the same body area. It is interesting to note that much time, energy and money are expended in the elimination of errors of as little as one one-hundredth this magnitude from basal metabolic rate determinations. To overcome these inaccuracies several interpolations, in addition to the original one by Aub and DuBois, have been made.

Some of these interpolations have been made by centering the stated value in the decades and the two-year periods. Others place the stated value at the zero, or one, or beginning of the decade, and still others at the nine, or ten, or end of the decade. These interpolations are made graphically on cross-section paper or by actual mathematical calculation by the usual method of successive differences.

Several variations and extensions of the original Sage standards have been used. Means and Woodwell[3] showed that, if from each value in the Sage standards there be subtracted 1.8 calories, results are obtained which are, on the average, practically identical with those obtained from the Harris and Benedict values. They not only did not recommend so changing the Sage standards but definitely recommended retaining them unchanged. Sanborn,[4] however, introduced this unrecommended standard into his percentile system of calculation of basal metabolic rate issued with instructions for the use of his respiration apparatus.

The data card accompanying the metabolimeter extends the Sage values so as to cover ages six to fourteen years by rather irregular two-year steps. Krogh has recently recommended the Sage standards reduced by 6 per cent.

The values of the Sage formula for surface area are given in the graph appearing in the original publication. Tabular arrangement of the values are given in No. VIII of this series of notes.

Harris and Benedict Standards.—Harris and Benedict,[5] considering the surface area law to be at best but an empirical formula, correlated twenty-four hour heat production upon direct physical measurements. They presented formulas, derived statistically from a series of normal subjects, to the values of which the individuals of the series conformed with least error when correlated in relation to sex, weight, stature and age. For convenience in use they presented tabular values of these formulas for individuals between twenty-one and seventy years old. Since the original communication Benedict has stated the formula for males to be applicable to subjects from one year to old age. Females below eighteen years old, however, show considerable variation from the values of the formula for adults.

Dreyer Standards.—Dreyer,[6] analyzing the data used by Harris and Benedict in deriving their formulas, showed that stature is not a legitimate biometric measurement upon which to correlate heat production. He derived statistical formulas from the values of which the variation of individuals of this normal series is less than from the values of the formulas of Harris and Benedict, since the biometric measurement, stature, is omitted. Correlation of heat production was made wholly upon sex, age, and actual weight. Dreyer, in the original preliminary communication, stated that still closer correlation would be obtained by substitution for actual weight, a theoretic normal weight based upon his formulas correlating weight with resting chest circumference and sitting height. The original Dreyer formula is probably more accurate when actual than when theoretic weight is employed; and when theoretic weight is employed, some modification of the formula should be applied.

Hobson,[7] in Dreyer's laboratory, writes that since "the measurements necessary to determine the 'normal (theoretic) weight' of the individual of Benedict's series were not available and the values published in his (Dreyer's) formula were derived from the observed weight; it is possible that these values may require some slight modification." He correlates the daily heat production of 46 normal men with age and theoretic weight based upon sitting heights and chest circumferences and concludes that for individuals leading a healthy active life, Dreyer's formula should be modified by approximately 2.5 per cent.

It may be concluded, therefore, that theoretic weight based upon trunk length and chest circumference has no advantage over actual weight in predicting heat production by Dryer's original formula. Tabular values of this formula for males from ages five to eighty have been published.[8] The values of the formula for females are 10 per cent below the corresponding values for males.

Standards for Children.—The present standards of heat production for the calculation of basal metabolic rates of children are even more widely divergent and unsatisfactory than the standards for adults. Probably the most used are those of Aub and DuBois for children not under fourteen years old, of Benedict[9] for girls twelve to eighteen years old, of Benedict and Talbot[10] for girls under twelve years, and of Harris and Benedict for boys of all ages over one year.

Dreyer and Hobson state that the Dreyer formulas are applicable to all individuals except those under five years old. It is interesting to note that as Dreyer ignored stature in his formulas for adults so did Benedict and Talbot not consider this biometric measurement to be related to heat production of children of certain ages.

VARIATIONS IN RESULTS

Much uncertainty exists in the literature concerning the magnitude of the variations in the results of basal metabolic rate determinations by employing the various standards.

Several series of determinations have been reported in which the basal metabolic rates were calculated, using both the Aub and DuBois standards and the formulas of Harris and Benedict. Among these are the series of Boothby and Sandiford,[11] who report 404 comparative tests in which the rates by the Harris and Benedict method are 6.5 points above those by the Aub and DuBois method, 455 comparative calculations substantially confirming this. Means and Woodwell find 6 points deviation in the same direction. In another series[8] of 350 routine hospital determinations, the Harris and Benedict standards yield results 3 per cent above the Aub and DuBois standards and the Dreyer formulas give rates 0.3 per cent above those of Harris and Benedict.

Very few comparative series have been reported using the Dreyer formulas, probably because of the difficulty of calculation.

CHOICE OF STANDARDS

Three main factors determine the choice of basal metabolism standards—first, custom; second, scientific foundation; and third, availability.

The older metabolism clinics adhere conservatively to the standards that have been used since their beginning thus making all their results comparable, while the younger clinics, having few or no comparative data on file, lose nothing by adopting the newer and possibly more scientifically based standards of comparison.

Those to whom the arguments of Harris and Benedict concerning surface area appeal use these standards in preference to those of Aub and DuBois, while those to whom Dreyer's argument against stature appeals employ his formula.

The Aub and DuBois standards are the most available of the three main systems. The table of values in calories and the height-weight graph of surface area have been reproduced in numerous articles and monographs.

The Harris and Benedict tables of values are available in the Carnegie Institution monographs, in Benedict's contribution to Abderhalden's Handbuch and in Joslin's[14] textbook. In consequence these standards are used less than those of Aub and DuBois.

The formulas of Dreyer, requiring logarithmic solution, have not been extremely popular among the rank and file of clinicians. Values of the male formula have been tabulated and published in Nos. V and VI of this series of notes, so that these standards need no longer be shunned on account of the mathematical difficulty. In fact, with these tables available, the Dreyer values are the simplest of any to apply to the determination of basal metabolic rate. Each of the others requires the use of two factors selected from tables or graphs while the value of Dreyer's formula is taken directly from a table as a single number. Another advantage of the Dreyer formula is that it is applicable in unchanged form to all ages from five to senility. Only one table of values is required for males and females as the values for females may be derived from those for males by deducting 10 per cent.

IMPORTANCE OF CHOICE OF STANDARD

It is becoming more and more generally accepted that basal metabolic rate determinations are of less value in diagnosis than in following the prog-

ress of treatment—surgical, radiologic, medicinal or hygienic. For this reason the importance of the present unsatisfactory nature of metabolism standards is minimized. Even though by the various standards one does obtain widely variant results from the same experimental data, in following the course of one particular case, if the same standard be used in successive estimations, changes in the metabolic rate may be satisfactorily estimated. For the purpose of following the course of treatment of a case it is not necessary to use any standard of comparison. The basal metabolic rate may be expressed, for such individual comparative determination, as total calories per hour or day, or in still simpler terms, as the oxygen consumption per minute, hour or day. Certain clinics have recently taken a step in this direction by expressing metabolic rate, not as a percentile variation of actual from expected normal heat production, but in actual calories per hour per square meter or in actual total daily calories. This leaves the diagnostic interpretation to the clinician's choice of standard and is perfectly satisfactory in interpreting the results of successive determinations in the study of the progress of an individual case under therapy.

The most important point in the whole subject of basal metabolism standards is for the clinician to know that there are more standards of comparison than for hemoglobin and that, just as in the case of hemoglobin studies, it is necessary for him to know the relative values of each in order to make proper diagnostic interpretations.

SUMMARY

The various present-day standards of reference for basal metabolic rate determinations of adults and children are outlined and compared. With a view to simplicity and uniformity, the Dreyer standards are recommended for the younger clinics without accumulated experimental data employing the older standards.

The variations between the results obtained from the various standards are less significant since the use of basal metabolic rate determinations has become almost wholly transferred from the field of diagnosis to that of therapeusis.

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WASSERMANN REACTION IN CEREBROSPINAL FLUIDS CONTAINING BLOOD*

By Alvin G. Foord, M.D., and Marjorie Bauckus,
Buffalo, N. Y.

RECENT literature and many of the standard textbooks of laboratory diagnosis contain references to the effect of blood in cerebrospinal fluid upon the usual tests for globulin[1] and the colloidal gold or other colloidal reactions, but its effect upon the Wassermann test has not been fully covered. Some technics described advise centrifuging the sample free from blood cells, inactivating the supernatant fluid, and conducting the test upon it. The theoretical objection to such practice is that small amounts of Wassermann-positive blood traumatically introduced by the spinal puncture needle into the cerebrospinal fluid may influence the Wassermann test thereof, since the volume of cerebrospinal fluid used in a Wassermann test is usually ten or more times the volume of serum usually used in standard technics. Also, sera reported as four plus by most qualitative technics are frequently much more potent than reported and many times will give four plus reactions when diluted ten times or more and, therefore, are really 40 plus or more (Table I). Consequently the Wassermann test on cerebrospinal fluid containing blood multiplies the effect of the use of large amounts of the fluid by the commonly high potency of the contained blood. This is particularly interesting in those cases in which the blood Wassermann is positive and a cerebrospinal fluid examination is made to determine the presence or absence of central nervous system involvement. In such cases cell counts are questionable when blood is present, even in microscopic amounts, and likewise the globulin and colloidal reactions are vitiated.

Our first experiments consisted of adding various small quantities of pooled Wassermann positive sera to pooled cerebrospinal fluid, negative in all regards (normal cell count, globulin and colloidal gold reaction, and negative Wassermann reaction), and determining the Wassermann reaction of the mixtures by a technic practically identical with that of the New York State Laboratory at Albany from which the antigens and amboceptor were received. The amounts tested with both the plain alcoholic and cholesterolized antigens were 0.4 c.c. and 0.2 c.c., the total volume of the test being 1.2 c.c. The mixtures were inactivated at 56° C. for twenty minutes before testing.

Table I shows that as little as 0.01 c.c. of positive serum in 2 c.c. of normal cerebrospinal fluid imparts enough Wassermann "reagin" to give a slightly positive test, and that as little as 0.04 c.c. will impart a four plus reaction to negative cerebrospinal fluid. Readings in salt solution were occasionally a little stronger.

*From the Laboratories of the Buffalo City Hospital.
Received for publication, March 28, 1927.

[1]Feinberg, S. M.: Value of Ross-Jones Test on Bloody Spinal Fluid, Jour. Lab. and Clin. Med., 1921, vi, 642.

TABLE I

AMOUNT OF SERUM (C.C.)	POSITIVE POOLED SERUM IN 2 C.C. NEGATIVE SPINAL FLUID												POSITIVE POOLED SERUM IN 2 C.C. NORMAL SALT							
	2/2/24		2/12/24		2/28/24		3/7/24		3/14/24		3/22/24		2/2/24		2/12/24		3/14/24		3/22/24	
	AL	CH	AL	CH	AL	CH	AL	CH	AL	CH	AL	CH	AL	CH	AL	CH	AL	CH	AL	CH
0.01	−	+	−	−	−	±	−	−	−	+	−	+++	−	++	−	+	±	++	+	+++
0.02	±	+++	−	+++	−	+	−	±	±	++	++	++++	±	+++	−	+++	+	+++	++	++++
0.03	+	++++	−	++++	−	++	−	+	++	+++	+++	++++	++	++++	±	++++	++	++++	++++	++++
0.04	+++	++++	+	++++	−	+++	−	++	++	++++	++++	++++	+++	++++	+	++++	+++	++++	++++	++++
0.05	++++	++++	+	++++	−	++++	−	+++			++++	++++	++++	++++	++	++++			++++	++++
0.06	++++	++++	++	++++	−	++++	−	++++			++++	++++	++++	++++	++	++++			++++	++++
0.07	++++	++++	++	++++	±	++++	−	++++			++++	++++	++++	++++	++	++++			++++	++++
0.08			++++	++++	+	++++	±	++++			++++	++++			+++	++++			++++	++++
0.09			++++	++++			+	++++			++++	++++			++++	++++			++++	++++
0.10																				
Control	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

AL = Plain alcoholic antigen.
CH = Cholesterolized antigen.

Next, instead of positive pooled sera, we used single fresh whole blood samples taken at random from forty serologically syphilitic patients, the vast majority with late lues, and added various quantities (from 0.005 c.c. to 1 c.c.) to 2 c.c. of negative cerebrospinal fluid. The amounts of blood added made the spinal fluids grossly bloody, those with the larger amounts decidedly so. Microscopic amounts were not used, because experience taught that such quantities of blood were of no importance. These were then freed of cells, and inactivated at 56° C. for twenty minutes before testing. Table II shows the results.

TABLE II

VOLUME SYPH. BLOOD ADDED	TOTAL TESTS	ALCOHOLIC ANTIGEN						CHOLESTEROLIZED ANTIGEN					
		–	±	1+	2+	3+	4+	–	±	1+	2+	3+	4+
c.c.													
0.10	36	12	3	3	6	4	8	0	0	4	6	6	18
0.07	3	1	0	2	0	0	0	0	0	1	1	1	0
0.05	29	15	4	4	1	4	1	4	3	5	7	4	6
0.03	12	9	1	0	2	0	0	4	3	1	1	2	1
0.02	12	9	1	2	0	0	0	7	3	1	1	2	1
0.015	5	5	0	0	0	0	0	1	0	2	2	0	0
0.010	7	6	1	0	0	0	0	2	0	0	3	2	0
0.005	3	3	0	0	0	0	0	2	1	0	0	0	0

The reactions (Table II) were less on the average than when serum was added, which was to be expected, since the serum constitutes roughly about 50 per cent of the blood volume. Partial fixation resulted in five out of seven tests when 0.01 c.c. of blood was added to 2 c.c. of negative cerebrospinal fluid; when 0.1 c.c. was added, complete or nearly complete fixation was noticed in over half of the tests (alcoholic antigen showed 3-plus or 4-plus in twelve out of thirty-six and the cholesterolized antigen in twenty-four out of thirty-six). Many of the patients from whom blood was drawn were under treatment, and if picked patients had been used instead of the ordinary run of hospital patients, the results, no doubt, would have been much more striking.

SUMMARY AND CONCLUSIONS

It has been demonstrated above that only very small amounts of Wassermann-positive blood need be introduced into normal cerebrospinal fluid to impart to the latter a positive Wassermann reaction, this being due to the fact that the test conducted with cerebrospinal fluid is done with relatively large amounts of fluid (ten times the usual serum amounts in most technics), and also because the usual four plus Wassermann sera are usually much stronger than four plus.

In examining cerebrospinal fluids from patients showing a positive blood Wassermann we strongly advise against the use of fluids containing traumatic blood in grossly visible amounts. Negative reactions are as reliable as in those not containing blood, but a positive reaction may be due either to neurosyphilitic disease or to the contained blood.

FROZEN SECTIONS, THEIR VALUE AS A ROUTINE PROCEDURE*

By Robert A. Keilty, M.D., Washington, D. C.

THE purpose of this paper is to present some of the facts in favor of frozen tissue sections as a routine procedure. Having had several inquiries lately as to the method in use, its importance might deserve a paper.

Frozen sections may be arbitrarily divided into two groups. First, those sections which are removed by biopsy for immediate diagnosis, and second, those sections which are fixed for a variable period and cut by the frozen method. The latter includes the routine surgical and autopsy sections.

The sectioning of fresh tissue removed by biopsy for immediate diagnosis has its field, and the advantage of this chance for diagnosis might be used more often than it is. I think one of the reasons for its lack of universal popularity is its infrequent use. Nobody about the laboratory is in practice, the apparatus is frequently out of order, the stains are not any too good and, of course, the finished sections show nothing but a poorly stained field full of artefacts. If for no other reason, practice in making frozen sections and apparatus in perfect order would be worth the effort to get good sections for biopsy diagnosis. In my own work I use exactly the same technic for fresh frozen sections as I do for the routine, staining with hematoxylin-eosin and not with polychrome methylene blue. There is no objection to the latter and where as many slides as are made in MacCarty's laboratory at the Mayo Clinic are examined by this stain I am sure one would like the picture quite as well as one made with hematoxylin-eosin. I prefer the latter stain, and while it makes the whole process a little longer—possibly five minutes—one obtains a good, well-cleared stain, and, being familiar with the stain, the diagnosis is much easier to make. The slides are permanent.

For routine work the fresh surgical specimen is fixed in 4 per cent formalin, i.e., 4 c.c. of 40 per cent formalin in 100 c.c. of distilled water. The whole specimen or a small section may be fixed. It should have plenty of fluid in a bottle large enough to hold it. Lightning top preserve jars will be found quite convenient for most specimens. Special care is necessary in putting up specimens removed by curettage. These are picked and placed together in a little pile on a single layer of gauze, and the loose ends of the gauze are held together with a small elastic band. The ball is made just tight enough to hold together. This is placed in the fixing fluid, and the whole, after fixation, is frozen and cut right through the gauze. The section when floated on water separates into the component parts of the scrapings,

*From the Laboratories of the Diagnostic Centre, U. S. Veterans' Bureau, Washington, D. C.

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and care is taken to have all types of small pieces mounted. This little trick overcomes about the only difficulty met with in freezing and cutting as far as the type of tissue is concerned.

Tissues are fixed in the formalin for twenty-four hours, very occasionally they may require a longer period but not often. At the end of this time they are frozen with carbon dioxide and cut. In freezing, a little practice determines just the proper time for cutting which usually lasts from about thirty seconds to a minute when all the sections necessary may be cut. Sections may be cut thin, but eight to ten microns will give good sections in most cases. One of the secrets of good sections is a sharp knife. Several knives should be kept on hand in perfect condition. It has been found that it is better to sharpen them immediately after using and put them away ready for the next time. The type of stone or strop used depends on the individual operator. I prefer a very fine oil stone followed by plenty of stropping. The Spencer automatic freezing microtome is very satisfactory.

With a little water the sections are floated from the knife into a small container of lukewarm water. The container should be black since it aids as a background to the white sections. All manipulation of straightening is done at this time in the water and none after it is mounted on the slide. For straightening in the water a small glass rod, a fine brush, or a very sharp-pointed steel needle may be used. After the section is spread out it is quickly mounted on the glass slide. If it is not smooth on the slide put it back in the water or take another section. Any straightening on the slide produces artefacts. The water is drained off. If the section is fresh frozen biopsy material, the slide is now flooded with 10 per cent formalin for one minute followed by 95 per cent and absolute alcohol, blotting after each fluid. If the tissue has been fixed the formalin is omitted. The alcohol is drained off, and the section is blotted with filter paper tight against the slide. It will spoil at this time if it is allowed to dry more than a few seconds. The section is then covered with three or four drops of thin celloidin in equal parts of absolute alcohol and ether, and with one blow the celloidin is evenly spread over the section. A little practice will make this perfect. If the celloidin is too thick it will diffuse the stain and mask the specimen, if it is too thin the section will come off in the stains; it is not hard to get it just right. The section is then ready to stain.

1. Stain, Delafield's hematoxylin; this should be old, ripe, and good and thick, and not frequently filtered. A good stain takes about two minutes. Use Coplin jars for all solutions. 2. Wash off excess in water. 3. Take out further excess in the section until light brown in 2 per cent hydrochloric acid alcohol, after fifteen seconds. 4. Develop blue color in ammonia water, about fifteen seconds. A few drops of pure ammonia water in a Coplin jar of distilled water. 5. Wash in water to clear ammonia. 6. Dehydrate with 95 per cent and absolute alcohol, a few seconds each. 7. Stain in saturated alcoholic eosin, if stain is right, about thirty seconds. 8. Clear out excess in two jars of 95 per cent alcohol and one of absolute. 9. Clear in carbol-xylol. 10. Clear out in two jars of plain xylol. 11. Mount in balsam.

With a little practice excellent sections can be obtained quickly. I have not cut a routine surgical or autopsy pathologic section in over four years by any method other than by frozen section, and this answers the question as to what type of tissue lends itself to this method. The answer is, all tissues.

Carbon dioxide tanks may be easily procured. It takes about four tanks, one in use, one in transit, one in stock, and one for emergeney. The tank in use should be placed horizontally above the freezing machine, the outlet end should be about two inches lower than the base. This obviates tank troubles. The ordinary tank cuts on an average of thirty-five specimens and costs about four dollars. Carbon dioxide may be used extensively for commercial ice packing in the near future. This will lessen this cost decidedly.

The big advantage in frozen sections is the rapidity with which they can be done; the fact that sections can be cut, examined, filed and reported out of the way at least the day following an operation. It gets the work out of the pathologist's hands promptly and into the surgeon's hands while the case is still fresh in his memory. As an example in autopsy work I recently had an autopsy at noon and presented the finished case with projection of the microscopic slides at the staff meeting the same evening. This was a decided advantage both to me and to the staff.

Frozen sections have no real disadvantage. There are many common objections, such as that the sections are too thick, the picture is incorrect, the stain is poor, or there are artefacts. None of these are real. The sections may be thicker than the two micron paraffin sections, but instead of being an objection this is often an advantage. One can see the whole cell and more especially one can get the true relationship of one cell to another without the par-boiled appearance of the paraffin. The picture instead of being incorrect is in reality the true picture of the individual characters of cell protoplasm and not the fixed hard picture of paraffin. By the method outlined the staining contrast of the hematoxylin-eosin is quite beautiful. Artefacts can be entirely eliminated by practice, and in only the delicate lacy textures of some structures, like the thyroid, will they be present. This can even be eliminated by a little longer primary formalin fixation.

SUMMARY OF METHOD FOR ROUTINE FIXED FROZEN SECTIONS

1. Fix small pieces of fresh tissue twenty-four hours in formalin.
2. Freeze and cut 8-10 microns.
3. Float sections onto warm water in dark container.
4. All manipulations should be delicately made in the water.
5. Mount section straightened out on a microscopic slide.
6. Drain off excess of water and blot with slight pressure.
7. Dehydrate 95 per cent, absolute alcohol, and blot dry.
8. Cover by one blow with a thin layer of thin celloidin.
9. Stain with hematoxylin, two minutes.
10. Wash off excess of stain in water.
11. Further excess of stain removed from section in acid alcohol, 15 seconds.
12. Develop blue color in very weak ammonia water, 15 seconds.
13. Wash out ammonia in water.
14. Dehydrate 95 per cent and absolute alcohol, few seconds.

15. Stain with saturated alcoholic eosin, 30 seconds to one minute.
16. Wash off excess of eosin in three changes of alcohol.
17. Clear in carbol-xylol.
18. Clear in two changes of plain xylol.
19. Mount in balsam.

Frozen sections are worth a try, and once one has seen their advantages one is wedded to them for routine purposes.

A MODIFICATION OF THE ORSKOV SINGLE CELL CULTURE TECHNIC*

By Esther Wagner Stearn, Ph.D., and Allen E. Stearn, Ph.D.,
Columbia, Mo.

INTRODUCTION

In 1922 Orskov[1] published a method for obtaining single cell cultures which is capable of rather easy application as compared with those previously described. Several changes in his technic, developed by us during the course of a series of investigations on bacterial variation, have led to a modified technic which is capable of results as clear-cut, and as reliable, as those obtained by using Orskov's original method, and yet which eliminates even more of the tedium of the older methods than does his, giving a simple method, easy of execution, and yielding practically unquestionable results.

OUTLINE OF METHOD

1. *Obtaining a Single Cell.*—Slides, marked as described below, are placed on a piece of filter paper in a Petri dish and sterilized in the oven in the usual manner. Clear centrifuged agar media is then allowed to flow on the slide from a sterile capillary pipette. For the successful preparation of a level agar surface four conditions must be fulfilled:

1. A high dilution of nutrient agar should be used in place of the usual concentration of agar. Diluted sugar agars may be used to advantage at times.

2. The agar solution, when poured, must be clear. It is therefore filtered and centrifuged.

3. A capillary pipette should be used to obtain a smooth flow and to give a thin layer of agar.

4. The glass slide should be hot during the pouring in order that the agar shall spread evenly in place of settling locally in lumps. Usually the pouring is done just after removing the slide from the sterilizing oven.

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*From the Department of Preventive Medicine and the Division of Physical Chemistry of the University of Missouri.

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[1]Orskov, J.: Method for the Isolation of Bacteria in Pure Culture from Single Cells and Procedure for the Direct Tracing of Bacterial Growth on a Solid Medium, Jour. Bacteriol., 1922, vii, 537.

A series of these plates is prepared at one time and placed in the refrigerator for from two to twenty-four hours for hardening. To insure the sterility of the agar, the plates are incubated for twenty-four hours and then examined.

A two-hour bacterial culture, which is much to be preferred to a twelve-hour one, is then used for streaking. The streaking is done with a very delicate platinum wire, which has been found to make the clearest lines. Streaking is preferred to spreading because of the increase in definiteness of the exact location of the organism. It has the added advantage that, as the colony under observation develops, there are only two directions to watch for the overlapping of other colonies from adjacent cells, in place of a large number. After streaking, the plates are incubated for from one-half to one hour, then examined, and then again incubated.

2. *Location of Isolated Organism and Relocation of Selected Organism or Colony.*—Diagrams, of the design shown by the continuous lines in Fig. 1, are scratched, using diamond on glass slides of good quality. After trying many different designs, this simple one was found to be best adapted and most adaptable to this method. The shifting of objectives, etc., often caused confusion when a more complex design was used.

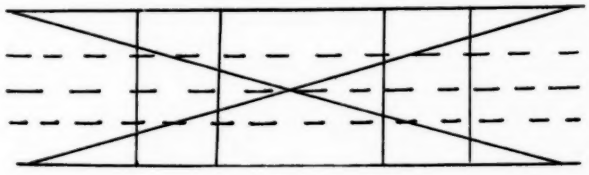


Fig. 1.

We have not found it necessary to use a micrometer scale attached to the eyepiece, as Orskov advises. The streaks, represented by the broken lines in Fig. 1, take the place of such a micrometer scale and make the "line pattern" for locating any particular colony a matter of certainty.

By means of the scales of the mechanical stage one can readily find the approximate position of a field on the diagrammed slide. The low power objective is used to obtain the exact position by focussing on a certain angle produced by the lines of the diagram, i.e., the pattern made both by the streaks and the lines scratched on the slide, and this "line pattern" can then be noted. An exact drawing of the diagram of the slide and streaks is made even before placing on the microscope stage.

The high power objective is used to locate the presence of isolated organisms along any streak. These are marked on the drawing and thus several single organisms can be definitely noted on the same slide.

After finding and marking the positions of the isolated organisms, the slide is removed from the stage, the ends passed swiftly through a flame, and it is then returned to the Petri dish and incubated. After incubation for varying periods of time the positions of the isolated colonies can be compared with those of the originally isolated cells. The filter paper in the Petri dish in which the slides are placed for incubation should be kept slightly moist.

3. *Subculturing from the Single Cell Colony.*—With plates which have been properly streaked it is always possible to locate colonies at a sufficient distance from adjacent ones so that transfers to tubes can be made directly from the marked colony in the ordinary manner. It is then merely necessary to note, under the low power of the microscope, the colonies next adjacent on either side to make sure that they have not been disturbed.

DISCUSSION

The above-mentioned modifications of Orskov's original method were adopted, not due to greater reliability of results obtained by their use, but because of the more readily acquired facility of manipulation and a smaller expenditure of time.

The principal differences between the modified and the original Orskov method are outlined below:

1. By use of centrifuged, very soft agar, poured by means of a capillary pipette directly on a sterile *hot* slide, thin, even layers of agar are obtained, thereby meeting Orskov's objection to the use of this method, and at the same time eliminating the necessity for his technic of transferring an already hardened agar slab from a Petri dish. The authors have found that this latter procedure is difficult because of the tendency of strips of agar which are sufficiently thin for the best results to curl and fold. When strips thick enough to be easily transferred in this manner are used, the images are blurred.

2. The use of the diagram shown in Fig. 1 is much simpler both for locating a precise area and for mapping a complete slide with respect to the location of the suitable single cells, than the more complicated patterns used by Orskov.

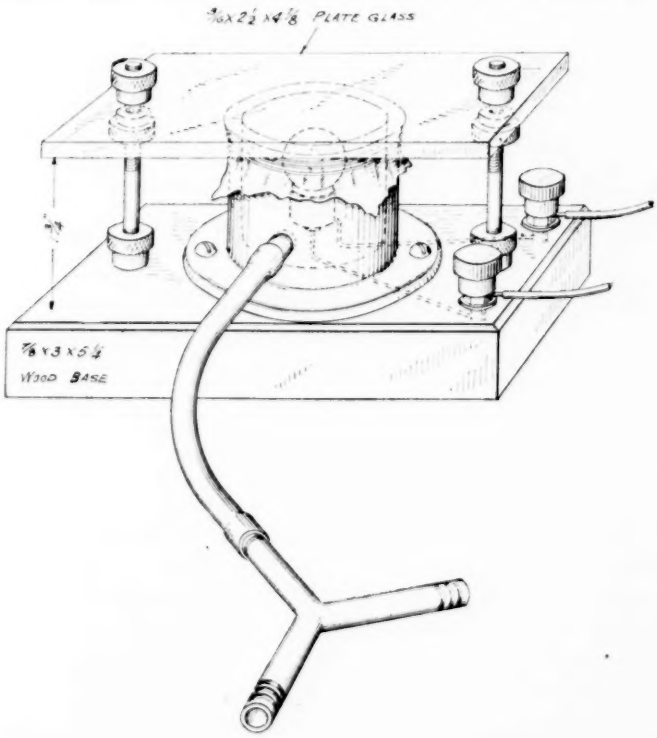
3. Streaking, rather than spreading, the inoculum overcomes certain disadvantages. Orskov states that some workers will prefer to streak, but he does not point out in any way its possible advantages, nor does he seem to consider it in any light other than as a possible alternative, with little to give either it or spreading any preference. In the first place streaking eliminates the necessity for the micrometer in the eyepiece, the "line pattern" being obtained by the superposition of the streaks on the pattern scratched on the slide. In the second place it reduces enormously the number of directions one has to watch for contaminating growth during the development of the single cell colony. One can always locate the streak, and it is a very simple matter to follow it until the organisms become sufficiently far apart so that, when they develop into colonies, transplants can be made from one of these without resorting to the somewhat tedious microscopic technic given by Orskov.

SIMPLE APPARATUS FOR REPEATED BLOOD PRESSURE DETERMINATIONS IN RABBITS*

By Theodore L. Squier, M.D., Milwaukee, Wis.

WHEN repeated blood pressure determinations in rabbits are required, a simple nonsurgical method of making the determination is of great assistance. The apparatus I have used is a modification of that described by H. C. Anderson[1] and of that described by T. Kuraya.[2]

A brass cup 2 inches in diameter is mounted, as shown in the accompanying diagram, on a wooden base measuring 3 by 5¼ inches. The edge of



the cup is flanged, and a series of shallow grooves are cut around the upper rim to permit a rubber dental dam to be stretched loosely and tied in an air-tight manner over the cup. Inside the cup is a flash-light socket and lamp bulb with connection through the base block to binding posts at one end of the block as illustrated. A metal tube screwed into the wall of the brass cup provides connection with a short piece of rubber tubing which in turn, through

*From the Department of Preventive Medicine, A. O. Smith Corporation.
Received for publication, April 13, 1927.

the Y-connection shown in the diagram, leads through one arm to an ordinary mercury manometer and through the other to a hand bulb for applying air pressure.

Threaded metal posts at each end of the block support a plate-glass plate 2½ by 5 inches with holes drilled at each end which permit the plate to slip over the supporting posts. The distance between the lower surface of this plate and the upper surface of the rubber membrane covering the cup can be adjusted easily by means of the supporting thumb screws beneath the plate, and after adjustment the plate is held firmly in position by the thumb screws above.

TABLE I

ILLUSTRATIVE BLOOD PRESSURE READINGS OF NORMAL RABBITS

	RABBIT NO. 36	
Dec. 18, 25	64-68-66-66-68	Aver. 66 mm.
Dec. 21, 25	62-72-74-70-74	Aver. 70 mm.
Dec. 22, 25	56-60-56-58-56	Aver. 57 mm.
Dec. 24, 25	52-54-54-54-54	Aver. 54 mm.
Dec. 28, 25	60-60-60-58-58	Aver. 59 mm.
Dec. 29, 25	60-58-60-62-58	Aver. 59 mm.
Dec. 30, 25	60-68-66-66-66	Aver. 65 mm.
Dec. 31, 25	64-64-63-62-66	Aver. 64 mm.
Jan. 4, 26	62-58-60-62-60	Aver. 60 mm.
Feb. 17, 26	59-62-63-59-56	Aver. 60 mm.
	RABBIT NO. 65	
Mar. 13, 26	54-56-54-53-51	Aver. 54 mm.
Mar. 16, 26	57-56-58-62-58	Aver. 58 mm.
Mar. 18, 26	53-53-56-52-50	Aver. 53 mm.
Mar. 22, 26	58-59-59-57-58	Aver. 58 mm.
Mar. 24, 26	56-58-56-60-58	Aver. 57 mm.
Mar. 26, 26	58-63-62-60-60	Aver. 61 mm.
Mar. 29, 26	66-65-64-60-65	Aver. 64 mm.
Apr. 3, 26	53-54-58-54-54	Aver. 55 mm.
Apr. 6, 26	66-65-64-63-63	Aver. 64 mm.
Apr. 7, 26	56-56-56-56-58	Aver. 56 mm.
	RABBIT NO. 152	
Apr. 12, 26	66-66-66-65-68	Aver. 66 mm.
Apr. 14, 26	66-66-68-66-65	Aver. 66 mm.
Apr. 17, 26	60-65-61-60-62	Aver. 62 mm.
Apr. 19, 26	63-64-62-60-60	Aver. 62 mm.
Apr. 23, 26	60-60-64-64-58	Aver. 61 mm.
Apr. 26, 26	63-64-66-66-66	Aver. 65 mm.
Apr. 28, 26	63-61-65-61-62	Aver. 62 mm.
Apr. 30, 26	68-70-68-70-70	Aver. 69 mm.
May 3, 26	67-69-69-65-69	Aver. 68 mm.
May 5, 26	67-66-70-66-70	Aver. 68 mm.

Blood pressure readings are made in the central artery of the rabbit's ear, the right usually being used because of greater convenience in handling. The rabbit is prepared by shaving the hair from the central part of the ear chosen. The glass plate is adjusted so that it will just clear the ear surface without exerting pressure. The rabbit is held in position with the left hand, and the upper screws are adjusted so that the glass plate is held firmly. Thus, the right hand is free to manipulate the manometer bulb. The pressure within the cup is gently increased until the blood flow in the central artery is just stopped. This is most easily observed through a simple tripod lens resting on

the glass plate. The mercury level in the manometer is then recorded. As a routine I take five readings and record the average of these as the pressure.

The following simple precautions should be observed:

1. The rabbit should be handled gently and care taken to prevent fright or excitement.

2. Approximately the same portion of the central artery should be used in making each subsequent reading.

3. The temperature at which the observations are made should be fairly constant.

It is to be recognized that the pressure reading so obtained is considerably lower than that obtained in the carotid by the surgical method.

In Table I are shown examples of blood pressures obtained as described. The apparatus has now been used for about two years and the method seems simple and accurate for purposes of comparative blood pressure study in rabbits over an indefinite period of time.

REFERENCES

[1]Anderson, H. C.: Demonstration of an Instrument for Taking Repeated Blood Pressures in Rabbits, With Report of Some Experiments, Proc. Soc. Exper. Biol. and Med., 1923, xx, 295.

[2]Kuraya, T.: New Nonsurgical Method for Blood Pressure Measurement of Rabbit, With Special Reference to Blood Pressure in Pericarditis; First Report, Acta. scholae med. univ. imp. Kioto, 1924, vi, 373.

STUDIES IN LOCAL ANESTHESIA. VII

The Toxicity of Some Derivatives of Para-Amino Benzoic Acid*

By Jacob Sacks, Ph.D., Chicago, Ill.

THE experiments reported here were carried out to determine the toxicity of a series of derivatives of para-amino benzoic acid. The compounds were prepared by Dr. Roger Adams of the Department of Chemistry of the University of Illinois. The anesthetic value of these drugs on the rabbit's eye has been reported by S. J. Cohen.[1]

Following are the names and formulas of the compounds in this series.

The method used to determine the toxicity was the intraperitoneal injection of the drugs into albino rats. The rats were weighed and the desired volume of a freshly prepared 1 per cent solution of the drug in 0.85 per cent sodium chloride solution injected into the peritoneal cavity of the rat, the animal immediately released, and the time of injection noted. The symptoms observed were essentially those reported by McGuigan and Brough[2] on another series of these derivatives, except in the case of those derivatives containing a cyclohexane ring (Nos. 22 to 28). With this exception, the following order of symptoms was noted: there was a period of hyperirritability,

*From Laboratory of Pharmacology and Therapeutics, University of Illinois College of Medicine.

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TABLE I

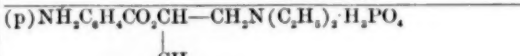
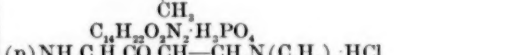
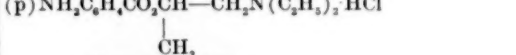
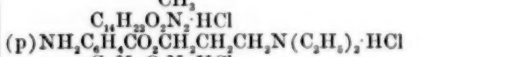
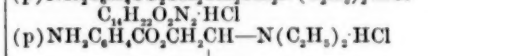
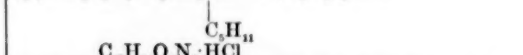
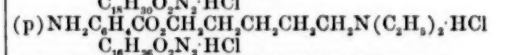
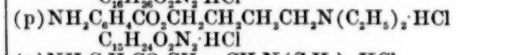
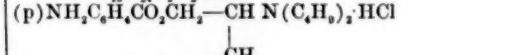
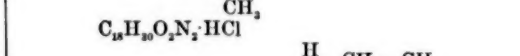
NAME	FORMULA	MOL. WT.
1.A. β-Diethylamino isopropyl para-amino-benzoate phosphate		348
1. β-Diethylamino isopropyl para-amino-benzoate hydrochloride		286
2. β-Diethylamino-n-propyl para-amino-benzoate hydrochloride		286
3. β-Diethylamino-n-heptyl para-amino-benzoate hydrochloride		342
4. ε-Diethylamino-n-amyl para-amino-benzoate hydrochloride		314
5. δ-Diethylamino-n-butyl para-amino-benzoate hydrochloride		300
6. β-Di-n-butyl amino-n-propyl para-amino-benzoate hydrochloride		342
7. γ (3-carbomethoxy piperidyl)n-propyl para-amino-benzoate hydrochloride		356
8. γ-Piperidyl-n-propyl para-amino-benzoate hydrochloride		298
9. γDi-iso-amylamino propyl para-amino-benzoate hydrochloride		370

TABLE I—CONT'D

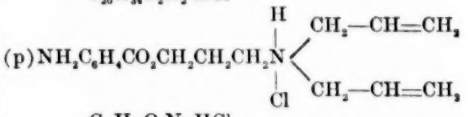
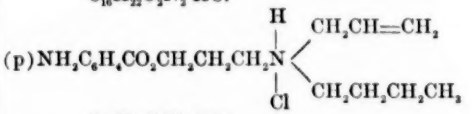
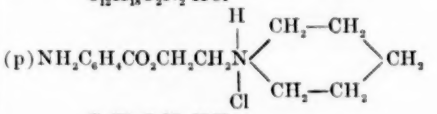
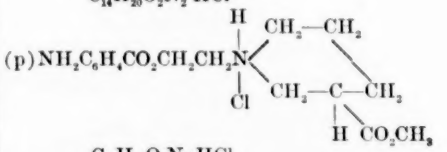
NAME	FORMULA	MOL. WT.
10. γ-Di-n-amylamino-propyl para-amino-benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2CH_2N(C_5H_{11}(n))_2HCl$ $C_{20}H_{34}O_2N_2 \cdot HCl$	370
11. γ-Di allylamino propyl para-amino-benzoate hydrochloride	 $C_{16}H_{22}O_2N_2 \cdot HCl$	310
12. γ-Allyl-n-butylamino propyl para-amino-benzoate hydrochloride	 $C_{17}H_{26}O_2N_2 \cdot HCl$	326
13. γ-Di-sec-butylamino propyl para-amino-benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2CH_2N(C_4H_9(sec))_2 \cdot HCl$ $C_{18}H_{30}O_2N_2 \cdot HCl$	362
14. γ-Dimethylamino propyl para-amino-benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2CH_2N(CH_3)_2HCl$ $C_{12}H_{18}O_2N_2 \cdot HCl$	258
15. β-Piperidyl ethyl para-amino-benzoate hydrochloride	 $C_{14}H_{20}O_2N_2 \cdot HCl$	284
16. β (3-carbomethoxy piperidyl) ethyl para-amino-benzoate hydrochloride	 $C_{16}H_{22}O_4N_2 \cdot HCl$	342
17. β-Di-iso-amylamino ethyl para-amino-benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2N(C_5H_{11}(iso))_2HCl$ $C_{19}H_{32}O_2N_2 \cdot HCl$	356
18. β-Di-n-butylamino ethyl para-amino-benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2N(C_4H_9(n))_2HCl$ $C_{17}H_{28}O_2N_2 \cdot HCl$	328

TABLE I—CONT'D

NAME	FORMULA	MOL. WT.
19. β-Di sec-butylamino ethyl para-amino-benzoate hydrochloride	(p)$NH_2C_6H_4CO_2CH_2CH_2N(C_4H_9(sec))_2HCl$ $C_{17}H_{28}O_2N_2 \cdot HCl$	328
20. β-Di-n amylamino ethyl para-amino-benzoate hydrochloride	(p)$NH_2C_6H_4CO_2CH_2CH_2N(C_5H_{11}(n))_2HCl$ $C_{19}H_{32}O_2N_2 \cdot HCl$	356
21. β-Allyl-n-butylamino ethyl para-amino-benzoate hydrochloride	(p)$NH_2C_6H_4CO_2CH_2CH_2N(H)(Cl)(CH_2{-}CH{=}CH_2)(CH_2CH_2CH_2CH_3)$ $C_{16}H_{24}O_2N_2 \cdot HCl$	312
22. 4-Dimethylamino cyclohexyl para-amino-benzoate hydrochloride	(p)$NH_2C_6H_4CO_2CH$ $<$$CH_2{-}CH_2$ $>$$CH\ N(CH_3)_2 \cdot HCl$* $CH_2{-}CH_2$ $>$ $C_{15}H_{22}O_2N_2 \cdot HCl$	298
23. 4-Dimethylamino cyclohexyl para-amino-benzoate hydrochloride	(p)$NH_2C_6H_4CO_2CH$ $<$$CH_2{-}CH_2$ $>$$CH\ N(CH_3)_2 \cdot HCl$* $CH_2{-}CH_2$ $>$ $C_{15}H_{22}O_2N_2 \cdot HCl$	298
24. 3-Dimethylamino cyclohexyl para-amino-benzoate hydrochloride	(p)$NH_2C_6H_4CO_2CH$ $<$$CH_2{-}CH_2$ $>$$CH_2$ $CH_2{-}CH$ $>$ $N(CH_3)_2 \cdot HCl$ $C_{15}H_{22}O_2N_2 \cdot HCl$	298
26. 3-Dimethylamino cyclohexyl para-amino-benzoate hydrochloride	(p)$NH_2C_6H_4CO_2CH$ $<$$CH_2{-}CH_2$ $>$$CH_2$ $CH_2{-}CH$ $>$ $N(C_2H_5)_2 \cdot HCl$ $C_{17}H_{28}O_2N_2 \cdot HCl$	326
28. 2-Dimethylamino cyclohexyl para-amino-benzoate hydrochloride	(p)$NH_2C_6H_4CO_2CH$ $<$$CH_2{-}CH_2$ $>$$CH_2$ $CH{-}CH_2$ $>$ $N(C_2H_5)_2 \cdot HCl$ $C_{17}H_{28}O_2N_2 \cdot HCl$	326

*Stereoisomeric forms. No. 22 has a melting point of 220°-222° C., while No. 23 melts at from 263°-264° C.

TABLE II

NUMBER OF COMPOUND	SMALLEST DOSE FOR CONVULSIONS, MG. PER KG.	AV. TIME TO ONSET OF CONVULSIONS AT DOSE STATED		M. L. D. MG. PER KG.	AVERAGE TIME TO DEATH		NO. RATS USED	NO. DEATHS
		MIN.	SEC.		MIN.	SEC.		
1A.	300	10	50	325	7	20	4	4
1.	150	9	00	275	8	35	6	3
2.	250	12	45	275	6	50	4	3
3.	75	4	15	125	6	05	2	2
4.	75	7	05	75	11	50	5	5
5.	50	6	20	75	13	35	6	5
6.	75	3	15	100	5	25	2	2
7.	350	11	30	400	21	40	2	1
8.	15	6	05	20	8	35	5	3
9.	75	2	00	75	6	15	2	2
10.	50	5	35	75	6	55	3	2
11.	100	3	40	100	6	00	5	3
12.	75	4	40	100	5	25	6	6
13.	75	4	50	75	7	55	2	2
14.	400	6	20	400	13	05	3	3
15.	150	3	50	175	7	20	4	4
16.	250	3	10	275	4	00	4	4
17.	75	4	40	100	8	30	2	2
18.	75	3	55	75	5	20	9	5
19.	100	2	35	150	6	35	3	3
20.	100	7	15	125	7	40	2	2
21.	75	7	50	90	4	20	5	3
22.	75	9	25	100	15	05	6	4
23.	50	5	25	75	6	45	4	4
24.	50	3	45	75	8	35	3	3
26.	50	2	50	75	6	15	1	1
28.	100	3	05	150	8	05	4	4
Tutocaine	100	8	45	125	6	15	1	1
Cocaine	50	6	05	50	9	00	4	2

during which a touch on the tail would cause a marked jump. Following this, there was a paralysis of the extremities; the respiration then became labored, the head was slowly drawn backward and the animal suddenly went into a clonic spasm. This passed off to be followed by another spasm after a short interval. During the convulsions, the animal was generally in opisthotonos. Between convulsions, there was usually a relaxation.

After a series of convulsions there would follow a period of coma, then death, by respiratory paralysis. The heart generally continued to beat for about a minute after respiration stopped.

Animals which did not die within twenty minutes were generally fully recovered within an hour after the injection.

The symptoms evoked when the cyclohexane derivatives were used were somewhat different. After the period of hyperirritability, there followed posterior paralysis, but without loss of equilibrium. The animal would then suddenly go into a convulsion, during which it was in the "Kangaroo" position, i.e., the fore-legs were elevated and flexed, and the body supported by the hind legs. Equilibrium was generally lost after the first spasm. Death in these cases was also by respiratory paralysis.

Unlike the effects of sublethal doses of the other members of the series, the effect of these derivatives did not wear off for several hours. There was a marked hyperirritability for as long as six hours.

TABLE III
COCAINE COEFFICIENTS

NUMBER OF COMPOUND	ANESTHETIC VALUE	TOXITY
1A.	0.19	0.15
1.	0.67	0.18
2.	0.86	0.18
3.	0.74*	0.40
4.	0.86	0.67
5.	0.71	0.67
6.	1.07*	0.50
7.	0.00	0.125
8.	0.67	2.50
9.	1.67†	0.67
10.	2.12†	0.67
11.	0.67	0.57
12.	0.67	0.50
13.	1.16*	0.67
14.	0.00	0.125
15.	0.24	0.29
16.	0.00	0.18
17.	2.38†	0.50
18.	0.69	0.67
19.	0.79*	0.33
20.	2.38†	0.40
21.	0.88	0.55
22.	1.02	0.50
23.	1.04	0.67
24.	0.52	0.67
26.	1.00*	0.67
28.	0.50	0.33
Tutocaine	----	0.40

*Irritant. †Corrosive.
Anesthetic value from data by Cohen.[1]

Table II gives the smallest dose causing convulsions, the average time to the onset of convulsions with this dose, the minimum lethal dose, the average time to the death of the animal with dose, the number of rats used and the number of deaths at this dose. Cocaine and tutocaine are included for comparison. The toxicity of procaine as determined by this method is reported by McGuigan and Brough.[2]

Table III gives the efficiency of the drugs in producing anesthesia of the rabbit's eye, with the duration of anesthesia with 1 per cent cocaine as unity, and the toxicity compared to cocaine as unity.

DISCUSSION

In those compounds of the series in which the aminoalcohol portion of the molecule is purely aliphatic (Nos. 1 to 6, 9 to 14, and 17 to 21), the minimum lethal dose is 75 to 100 mg. per kg. of body weight in eleven of the seventeen compounds. Some of the corrosive ones, those with 4– and 5–carbon alkyls, have no greater toxicity than those which produce good anesthesia.

Of the remaining six purely aliphatic derivatives, two of high molecular weight, Nos. 3 and 19, have a somewhat lower toxicity. No. 14, which is of low molecular weight and has no anesthetic value, has a correspondingly low toxicity.

Compounds Nos. 1 and 2 show a high anesthetic value combined with a remarkably low toxicity. No. 1 is an isopropyl derivative and No. 2 a normal propyl derivative with the diethyl-amino group in the β-position.

Those compounds containing a piperidine ring (Nos. 7, 8, 15 and 16) show marked concordance between anesthetic value and toxicity. No. 8, which is an effective anesthetic, is very toxic, No. 15 is less effective and less toxic, and the other two have no anesthetic value and very low toxicity.

The cyclohexane derivatives (Nos. 22 to 28) show better anesthetic powers than the purely aliphatic ones, but their toxicity is of the same order.

REFERENCES

[1]Cohen, S. J.: Jour. Lab. Clin. Med., 1927, xii, 983.
[2]McGuigan, H. A., and Brough, G. A.: Jour. Lab. Clin. Med., 1925, ii, 479.

A PRACTICAL TECHNIC IN THE PREPARATION OF SMEARS FOR THE EXAMINATION OF TUBERCLE BACILLI*

By L. T. Black, M.D., Denver, Colo.

THE old technic used in the examination of smears for the tubercle bacilli and in making comparisons of different smears according to the Gaffky scale is not only of no value for clinical purposes, but very often has a detrimental psychologic effect on the patient. A patient feels that, if the reading of his Gaffky scale is 7 per field today and was only 4 per field four weeks previously, he is clinically worse.

What are the mistakes made according to the old method? We hunt for lentil-like particles in the sputum and smear it thickly on the slide. Sometimes we find tubercle bacilli in those particles and sometimes we do not, and it very frequently happens that our laboratory diagnosis is "too many to count" in a case which, from a clinical and x-ray standpoint, must be considered as greatly improved. On the other hand, we find fewer bacilli in the smear of a patient who, from the clinical standpoint, is definitely worse. In other words, we fool not only the patient but also ourselves.

During the past two years we have been using a technic suggested by Dr. Felix Baum, our Medical Director. Dr. Baum had used this method for many years in Europe and had had very satisfactory results. The technic is very simple:

The sputum is placed in a bottle or container and whipped for one minute with a wooden applicator around the end of which a piece of cotton has been wrapped. The tubercle bacilli present in the sputum will become adherent to the cotton. The cotton-wrapped end of the applicator is then drawn once very lightly and quickly across the slide, leaving a thin smear in which the tubercle bacilli are distributed homogeneously.

This method has been used in hundreds of cases and we have concluded that in many of the cases where we could not find tubercle bacilli according to the old method of smearing, the sputum was positive with this technic. The Gaffky scale applied to this technic is obviously of greater value in the determination of the degree of the tuberculous process.

*From the Medical Department, National Jewish Hospital, Denver, Colo.
Received for publication, April 18, 1927.

CORRESPONDENCE

Sir.—The report which I am about to make may possibly be interpreted as an attempt to discredit the work of one of your contributors. Please be assured that such is not the case, and that the only attempt made is to establish the scientific truth of the case, that factor that sometimes so easily eludes the best of us.

In an article entitled "A Study of the Antigen used in the Wassermann Test for Syphilis" in your issue of June, 1926, xi, No. 9, page 864, Mr. S. L. Leiboff, Ph.B., of New York in a consideration of the rôle of the antigen in the fixation of complement and in the formation of a precipitate, makes the following statement:

"Heating of the beef heart tissue or of the alcoholic extracts used as antigens in the Wassermann test for syphilis does not affect their complement fixation power but does destroy their precipitate-forming property."

On reading this article with interest, and having available a heat extracted antigen which I had prepared several months ago for use in the Kahn test, but which to date I had not used, I determined to check the above quoted statement in so far as it bears on the precipitate-forming property of an antigen for the Kahn test. With the correctness of the first part of the statement I was already familiar, having previously successfully used a heat extracted antigen in the Wassermann test.

A known positive syphilitic serum was obtained which had been reported four-plus positive Wassermann and four-plus positive Kahn by the Hygienic Laboratory of the State Board of Health at Columbia, S. C. This serum together with a known negative serum as control, was run through the Kahn three tube macroscopic technic, using both an antigen prepared according to the Kahn Standard formula, and the heat extracted antigen referred to above which will be described later. The result of the test was positive by both antigens in all three proportions. A microscopic examination of the precipitates formed showed slightly larger granules by the Kahn antigen, the heat extracted antigen precipitates being of smaller granules but more thickly distributed throughout the field.

In order to further test the heat extracted antigen the same two sera were run through with it by Kahn's presumptive procedure of high sensitiveness using a Kahn Special antigen for comparison. The results were identical with those of the three tube test.

In order to test the sensitivity of the heat extracted antigen the known positive syphilitic serum was run through by Kahn's quantitative procedure, using the heat extracted antigen and the Kahn Special for comparison. The serum showed a positive titer of 20 reacting units, being positive in the 1/5 dilution by both antigens, thus proving an equal sensitivity for the heat extracted antigen with the Kahn antigen. A microscopic examination of the contents of the tubes containing the next higher dilution of the blood serum showed no evidence of any precipitate by either antigen.

It would, therefore, seem that Mr. Lieboff's statement is in error, and that the heating of the beef heart tissue or of the alcoholic extracts used as antigens does not permanently destroy their precipitate-forming property. Mr. Leiboff's conclusions were based on experiments with the Sachs and Georgi reaction, and my findings may therefore be said to be only further evidence of the fact which I believe is now generally admitted, that Doctor Kahn has come closer to achieving a rational precipitation technic than any of his predecessors.

The heat extracted antigen referred to, which I have called the Penn antigen after its originator, Mr. R. H. Penn of the Laboratory Staff of the South Baltimore General Hospital, Baltimore, Md., is prepared as follows: 50 grams of dried, ground and pulverized fresh beef heart is extracted for three days in the refrigerator by ether, in a 500 c.c. Erlenmeyer flask, the ether being added to cover the heart tissue to a depth of one inch, and discarded at the end of each day and replaced by a fresh portion of ether. The heart

tissue is then again dried in air until all trace of ether is removed and extracted with ethyl alcohol, 5 c.c. per gram of beef heart, by boiling gently for one hour in a flask over a Bunsen flame, with an inverted funnel in the mouth of the flask to serve as a condenser, the loss of alcohol by evaporation being replaced; and further extracted by heating for one hour in a flask immersed in a bath of boiling water. The alcoholic extract thus obtained is cholesterolized to the extent of 4 mg. of cholesterol per c.c. of extract. The antigen thus prepared is one showing usually a very high titer for the Wassermann test, in which it has been used satisfactorily now for some time at the South Baltimore General Hospital. As titrated by the writer for the routine 3 tube Kahn test it showed best results in proportions of 1 plus 2.5 with physiologic salt solution. As used for the Kahn quantitative procedure experiment referred to above an arbitrarily chosen titer of 1 plus 1.5 was employed with apparently satisfactory results.

(Signed) ARTHUR T. BRICE, JR., B.A.

Florence, S. C., August 9, 1927.

Sir.—It appears to me that the different results obtained by A. T. Brice, Jr., and myself are due to two factors: one is the difference in the technic of the preparation of the antigen; and the other is, as Mr. Brice suggests, the use of a different method in performing the tests.

Since no statement to the contrary is made, I presume that in the "Penn" antigen the heart-muscle is dried at room temperature. In the antigen which I used the tissue was treated at high temperature (JOUR. LAB. AND CLIN. MED., 1925, xi, 127); the tissue was mixed with water and autoclaved at 15 pounds pressure for ten minutes; it was then strained through cloth, washed with hot water, and dried in an electric oven at 100° C. until perfectly dry. The dry powder was ground, completely extracted with ether and finally with boiling alcohol. Since I have not tried the Kahn test with this antigen, I am in no position to say how it would behave in that test.

The variations in the technic in the performance of the Sachs-Georgi and the Kahn tests as to the method of preparing the antigen, dilution, and incubation are factors which would greatly modify the results, particularly since we are dealing with colloidal substances.

That heat produces a deleterious effect upon the antigen used in the Sachs-Georgi test is indicated by the following statement of Sachs: (Arch. f. Dermat. u. Syph., 1921, cxxxii, 18) "mit den Vorwärmen bei höheren Temperaturen unspezifische Reactionen, die nach zweistündigen Aufenthalt in Brutschrank und nachfolgender Zimmertemperatur auftreten, sukzessive schwinden. Jedoch sind sie erst nach zweistundigem Vorwärmen bei 60° vollständig eliminiert, vobei aber die Reactionsstärke schon immerhin deutlich abgenommen hat."

The loss of precipitate-forming property by incubation at 50° C. cannot be ascribed to an alteration of the serum, for all sera are inactivated at 56° C.; and it was further brought out by Kahn, Johnson, and Boyd (Jour. Infect. Dis., 1921, xxix, 639) and by Kahn and Johnson (Ibid., 1922, xxxi, 438) that heating at 56° C. for three hours had very little effect on the reactive substances in syphilitic serum. The indication is that Sachs' results were due to some alteration of the antigen by the heat.

My interest in the Sachs-Georgi test was not per se, but in the question of whether complement fixation in syphilis did or did not depend upon the formation of a precipitate. I did not make use of the more delicate and more specific test of Kahn for the only reason that this work was started in 1921, before the Kahn test had gained its present popularity.

In the article referred to by Brice, on page 862, I made the following statement: "Failure to notice a precipitate macroscopically does not prove the absence of an invisible precipitate: we know that albumin particles may aggregate into larger particles without causing precipitation, provided the excess of one of the precipitate-forming colloids acts as a protective colloid. Thus, further evidence is necessary to settle this question."

(Signed) S. L. LEIBOFF.

New York, Oct. 12, 1927.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LABORATORY TECHNIC

BLOOD, STAINING OF: Technic for Staining of Blood Preparations by Giemsa's Method, Van Walsem, G. C. Nederl. Tijdschr. v. Geneesk., January 23, 1926, i, 351.

From a study of factors concerned with the production of inconstant results obtained with Giemsa's blood stain van Walsem makes the following recommendations for its use:

1. The stain is best prepared in small quantities so as to be relatively fresh when used.

2. To prevent agglutination and facilitate staining the blood should be collected as described below.

A "separating fluid" of the following formula is prepared:

Chloral hydrate 4 per cent—8 parts
Cocaine hydrochloride 6 per cent—3 parts
Morphine hydrochloride 1⅓ per cent—2 parts
Glucose 3 per cent—13 parts

This solution is clear and stable. To seven parts add two parts of 0.5 per cent sodium carbonate solution. The resulting mixture, which is the "separating fluid," is turbid. One part of this solution is mixed with two parts of blood and smears made from the sediment after centrifugation.

3. After standing eight hours the slides are fixed for fifteen minutes in methyl alcohol, dried by centrifugation, immersed in 1 per cent chloroform for one minute, in methyl alcohol, and again dried.

4. Stain two hours.

5. Change to dilute stain (1 drop to 1 c.c.) for one hour.

6. Rinse, dry and differentiate two minutes in equal parts of cedar oil and oil of cloves.

7. Blot dry with filter paper and examine.

LIVER FUNCTION: Rose Bengal in Functional Examination of the Liver, Fiessinger, N., and Walter, H. Bull. et mem. Soc. Med. d. Hop. de Paris, April 1, 1926, xlii, 525.

After an examination of more than fifty cases, the authors arrived at the following conclusions:

The rose bengal test of liver function is a simple and easy method and is harmless.

It is a test which, while it does not permit the determination of the amount of insufficiency, is always very positive in marked affections of the liver and is negative in individuals with a normal liver. For this reason it is often a valuable aid in diagnosis. A finding of more than 3 mg. per liter of blood indicates the necessity of searching for an affection of the liver which frequently is latent.

From a point of view of prognosis, it is not of immediate value except as a simple function test when used along with tests of other functions.

When repeatedly made on one patient during the course of his disease it permits the exact determination of an affection of the liver parenchyma.

DIPHTHERIA: Pergola's Nutrient Substratum for Bacilli of Diphtheria, Nyfeldt, A. Ugesk. f. Laeger, February 18, 1926, lxxxviii, 165.

Nyfeldt conducted an extensive investigation as a result of which the suitability of Pergola's medium for B. diphtheria is confirmed.

The formula for Pergola's medium is:

Serum (ox, horse, goat)	50 c.c.
Sod. chloride solution 0.8 per cent	50 c.c.
Potassium tellurite solution 2 per cent	1 c.c.
Yolk of egg	1

This mixture is emulsified in a sterile flask and from 5 to 7 c.c. are placed in test tubes and coagulated as a slant for one hour at 90° C. and subsequently sterilized in the autoclave.

WASSERMANN: The Reactivation of the Wassermann Reaction and Infections, Milian, G. Bull. Soc. Med. Hop. Paris, May 27, 1926, xlii, 795.

A short time ago Dufour reported examples of reactivation of the Wassermann reaction following injection of typhoid vaccine. The author was much interested in the communication and believed that a history of this subject would be appropriate.

In 1910, the author designated under the term reactivation a phenomenon consisting in the appearance of the Bordet-Wassermann reaction after antisyphilitic treatment, especially 914, when this reaction appeared to be absent previously. He applied this test to the study of syphilis in general and particularly to the determination of cure from syphilis. It cannot be denied that in a patient who has had no treatment and whose reaction is negative, if the reaction becomes positive after a short treatment it is a proof that the patient was not cured, while a persistent negative reaction is an argument in favor of cure.

Since the discovery of this reactivation by 914, endeavors have been made to find better methods by which positives could more frequently be obtained. It was found that this could be done best by an incomplete treatment consisting of one or two injections. Other antisyphilitic remedies, such as mercury, iodine and bismuth, appeared to be capable of giving the same results.

But later it was found that nonspecific substances had the same power such as collargol, radium bromide and milk. Some, but not all, infectious diseases are capable of producing a reactivation. This phenomenon has been observed after herpes and scarlet fever.

Neuda has shown the influence of influenza on the Wassermann reaction. Marini has shown that typhoid fever has the same power. Other authors have found the same reactivating effect in typhoid or cholera vaccine and also in sterilized milk.

The author believes that these various agents do not cause a simple reactivation of the Wassermann reaction but that they really produce a recrudescence of the syphilis itself due to a biotrophic action of the chemical substance of the vaccine or of the bacteria on the parasite of syphilis.

WASSERMANN: On the Reactivation of the Wassermann Reaction in Experimental Infections, Dufour, H., Casteran, A., and Eidiez, B. Bull. Soc. Hop., May 20, 1926, xlii, 778.

For a long time the authors noticed that the Bordet-Wassermann reaction became positive in a certain number of patients who had a more or less general infectious disease. Thus many cases of hereditary syphilis show a positive reaction after a tuberculous pleurisy or an epidemic encephalitis.

Cases of cerebral tumors have been reported in which the Bordet-Wassermann reaction in the cerebrospinal fluid was positive. Such facts have led investigators to throw doubt on the Bordet-Wassermann reaction or to consider its presence as a proof of reactivation in latent syphilities.

Milian has shown that scarlet fever, herpes, x-rays, etc., reactivate the Bordet-Wassermann reaction in syphilities or hereditary syphilities.

The authors have endeavored by means of a local inflammation to reactivate the reaction in known syphilitics who had been negative. They used fixation abscesses but were not successful in producing a reactivation.

They then determined to try an experimental general infection and for this purpose used antityphoid vaccination. Two injections of this vaccine were given at eight day intervals to a man sixty years old who had been syphilitic since the age of twenty. The Bordet-Wassermann reaction was negative before the injection. When his temperature was 38° C. (100.4° F.) he gave a clearly positive reaction. Three weeks later the reaction again became negative. This is an experimental demonstration that an intercurrent general disease can reactivate the Bordet-Wassermann reaction in a syphilitic. It should be understood that all patients do not react in this manner. For example, a syphilitic with a negative reaction retained this negative reaction in spite of an attack of tuberculous peritonitis with considerable fever.

JAUNDICE: Excretion of Phenolsulphonephthalein in Obstructive Jaundice, Abramson, H. A. Arch. Int. Med., February, 1926, xxxvii, 291.

The phenolsulphonephthalein concentration was readily determined in the presence of the bile pigments by precipitating the extraneous pigment with an excess of saturated barium hydroxide. In urine containing bile pigments the dye is partially adsorbed during precipitation, the degree of adsorption being dependent on the concentration of pigments and total urine volume. The correction for adsorption is easily determined because the quantity of adsorbed phenolsulphonephthalein in a given specimen of urine whose pigments have been precipitated by barium hydroxide, as far as the accuracy of the test requires, is a constant within the limits of from 0 to 6 mg. (0 to 100 per cent) of the dye.

When there is no correction for adsorption:

1. It is preferable to give the dye intravenously but intramuscular injection is almost as suitable.

2. The two hour specimen should be divided into two equal parts.

3. To one part an excess (urine volume plus 50 c.c.) of saturated barium hydroxide should be added, and this should be diluted to 500 or 1000 c.c. and then filtered. A portion of the filtrate should be compared with standards. If the 500 c.c. dilution is used, the reading is direct. If 1000 c.c. is the dilution, the two hour excretion is the reading times two. It is, of course, only necessary to catch a few c.c. of the filtrate. The dilution to 2 liters aids in colorimetric comparison.

4. If the total two hour percentage of excretion of dye is normal or only 5 or 10 per cent below the lower limits of normal, the reading stands as the excretion of the dye and the excretion may be considered normal.

When correction for adsorption must be determined:

5. If the reading obtained is below normal, it must be determined whether the diminished excretion of the dye is actual or is due to adsorption. To the remaining half of the urine, of which the dye content has been determined, 0.25 c.c. (500 c.c. dilution) or 0.5 c.c. (for 1000 c.c. dilution) of phenolsulphonephthalein should be added. With intense jaundice and large urine volume the latter is preferable. The percentage of dye in this control should be redetermined.

6. The second reading in Paragraph 5 minus the first reading in Paragraph 4 gives the quantity of dye not adsorbed. For example, if the first reading (apparent two hour excretion) had been 20 per cent and if to the remaining portions 0.25 c.c. of the dye had been added, the second reading, granting that none had been lost by adsorption, would be 20 per cent plus 50 per cent (0.25 c.c. of dye to 500 c.c.) or 70 per cent. If the reading were 50 per cent instead of 70 per cent, it would be evident that the added dye gave only an additional 50 per cent minus 20 per cent, or 30 per cent of color. Hence, three-fifths of the dye added was determined and two-fifths lost by adsorption.

7. The final corrected reading of excreted dye is equal to the first reading divided by the fraction not adsorbed, or

$$\frac{20 \text{ per cent}}{3/5} = 20 \text{ per cent} \times 5/3 = 33 \text{ per cent.}$$

In the event that the colorimetric comparison is not good because the pigment has not all been removed, practically the last traces may be precipitated by making a second dilution using barium hydroxide instead of water as the diluent. The second dilution does not cause much adsorption of dye because the mass of precipitate is usually small. Furthermore, the colorimetric comparison with standards above 30 per cent has been difficult, and the most accurate readings are obtained by comparison with lower values and by then multiplying by the necessary correction factor.

Summary of Results and Comment.—The phenolsulphonephthalein excretion was found to be normal in fifteen cases of obstructive jaundice with a varied etiology. In all of these cases there was no evidence of renal insufficiency. The duration of jaundice was up to six months. Seven cases had periods of intense icterus lasting more than one month. Three of these lasted more than two months. The ages ranged from twelve to seventy-two years.

It would seem from the morphologic changes in the kidney in jaundice that the degenerative processes are to be found chiefly in the convoluted tubules. In uncomplicated obstructive jaundice, the excretion of phenolsulphonephthalein is normal. This normal excretion of the dye would fit in with the hypothesis that the excretion takes place through the glomeruli of the kidney, since there is apparently no evidence that the severe renal poisons that are present in jaundice influence the structure of the glomeruli.

These prolonged cases of jaundice demonstrate that in uncomplicated obstructive jaundice kidney function as estimated by the foregoing methods is probably unimpaired. What is the influence, then, of obstructive jaundice on diseased kidneys that are functioning normally but whose reserve is near the point at which additional injury may produce diminished function? Are the cases of obstructive jaundice which develop uremia those in which a further slight kidney injury leads to renal dysfunction? And finally the question arises, Is this injury simply due directly to the action of bile pigments and salts on the kidney, or is it due to a disturbance of the total metabolism?

Conclusions.—1. The excretion of phenolsulphonephthalein by the kidneys in obstructive jaundice has clinical and physiologic significance.

2. In fifteen cases of uncomplicated obstructive jaundice the excretion of phenolsulphonephthalein was found to be normal.

SMALLPOX: The Blood in Purpuric Smallpox, Ikeda, K. Jour. Am. Med. Assn., June 13, 1926, lxxxiv, 1807.

A clinical review of forty-eight cases. The principal changes were found in the cellular elements of the blood, involving the numerical values of the platelets and leucocytes and the morphologic structure of the leucocytes and erythrocytes.

A marked and progressive thrombopenia until its termination characterizes the purpuric smallpox in contradistinction to a rapid and steady rise in platelets after the vesicular stage in all other forms.

Another significant finding is a rapid decrease in the polymorphonuclear neutrophiles. All observers agree as to the presence of a lymphocytosis in smallpox.

Along with the appearance, in varying numbers, of myelocytes, young leucocytes, and atypical and pathologic lymphocytes, an absolute lymphocytosis of moderate degree was found in all forms of pustular smallpox.

In the purpuric type, however, a disappearance of the polymorphonuclear elements was effected so suddenly and so nearly completely that, with a very high leucocyte count, a leucemic state or acute benign lymphadenosis might be diagnosed from the blood alone.

Another striking condition was the appearance of pathologic forms of normoblasts, basophilic stippling, and polychromatophilia without clinical or laboratory evidence of severe anemia or chronic sepsis. These findings may be taken as indicative of intense bone marrow stimulation.

A most striking finding in the stained smear was the presence of fragments of condensed nuclear bodies, usually surrounded by a small portion of neutrophilic cytoplasm. These were derived from polymorphonuclear leucocytes.

These changes are held to be due to the direct effect of a toxin virus on the cellular structure of the mature leucocytes in the circulating blood and Ikeda believes them reasonably specific and occurring sufficiently early to make them of positive value in early diagnosis.

In the terminal stages an intense bacteriemia—frequently of hemolytic streptococci—was frequently encountered.

The hemoglobin and red cell count were practically normal.

Lymphocytes showed the same picture as seen in pustular smallpox: the presence of pathologic forms such as atypical lymphocytes, plasma cells, Turek's "irritation forms," and occasional immature forms. Monocytes showed no characteristic changes.

Myelocytes and metamyelocytes were frequently found. Eosinophiles and basophiles showed no changes. Erythrocyte fragility remained normal. Bleeding time seemed prolonged and coagulation tests were inconstant, though these observations were not recorded in a sufficient number of cases to permit conclusive impressions.

MEDIA: A Synthetic Food Medium for the Cultivation of Drosophilia, Pearl, R. Jour. Gen. Physiol., March 20, 1926, ix, No. 4, p. 513.

The following medium has been found to be well adapted to the cultivation of the fruit fly.

Solution A.	Cane-sugar	500	gm.
	$KNaC_4H_4O_64H_2O$	50	gm.
	$(NH_4)_2SO_4$	12	gm.
	$MgSO_47H_2O$	3	gm.
	$CaCl_2$	1.5	gm.
	H_2O to make 3000 c.c. of solution.		
Solution B.	Agar-agar	135	gm.
	Tartaric acid ($C_4H_6O_6$)	30	gm.
	KH_2PO_4	6	gm.
	H_2O to make 3000 c.c. of solution.		

Melt the agar thoroughly in the water with heat, add the salts, and for the medium to be used in the fly bottles, mix equal parts of Solutions A and B. For some kinds of work it has proved desirable to have the final food a little less stiff, in which case a smaller amount of agar is used, without changing the composition otherwise.

This medium has a P_H when freshly made, cooled, and the agar set, of approximately 3.7. As the flies live upon it the P_H falls to a value of 3.0, or in some cases even lower.

STAINS: Chemical Studies on Polychrome Methylene Blue, MacNeal, W. J., and Killian, J. A. Jour. Am. Chem. Soc., 1926, xlviii, 740.

A description of methods for the manufacture of dimethylene-blue chromate, methylene Azure B, methylene Azure A, and methylene violet, for the details of which the original paper should be consulted.

TUBERCULOSIS: Blood Cell Volume Index in Pulmonary Tuberculosis, Newcomb, P. B. Southwestern Med., March, 1926.

Using the Van Allem hematocrit, Newcomb reports a study of the blood cell volume in 108 cases of pulmonary tuberculosis as a result of which it is concluded that the blood cell volume index is advocated as an addition to the routine blood examination in pulmonary tuberculosis. It appears to be less liable to human error than the hemoglobin percentage and color index in that its computation is mathematically and mechanically controlled.

In a brief experience the blood cell volume index has been found to bear generally a true and consistent relation to the patient's condition—normal or high readings being the rule in those who are showing progress under treatment, while low values are recorded for patients who are failing physically.

BILIRUBIN: Comparison of the Sensibility of the Reactions of Gmelin and Grimbert for the Presence of Bilirubin in the Urine, Garnier, M., and Giroire, H. Paris, Med., May 15, 1926, xvi, 483.

Gmelin's reaction is much less delicate than the precipitation methods, especially that of Grimbert the technic of which is as follows: 5 c.c. of a 10 per cent solution of barium chloride is added to 10 c.c. of urine, the mixture is shaken violently and is then centrifuged. The precipitate consisting of sulphate, phosphate and bilirubinate of barium is first washed by centrifuging in 10 c.c. of water, then, after decantation, it is taken up in 4 c.c. of 90 per cent alcohol containing 5 per cent of its volume of pure hydrochloric acid. This mixture is placed on a boiling water-bath for about one minute. If the liquid is colorless there is no biliary pigment in the urine but if colored green or bluish green the urine contains bilirubin, if it is colored blue, violet or carmine red, the urine contains biliary pigments in the process of oxidation. Finally, if the liquid presents a brownish tint, two drops of hydrogen peroxide solution must be added and the mixture again is placed on the boiling water-bath; the appearance of a green color shows the presence of biliary pigment.

Grimbert's reaction is ten times as sensitive as Gmelin's reaction.

In certain urines, Gmelin's reaction is not obtained as the colors are masked by other colored substances in the urine but Grimbert's reaction is positive if biliary pigments are present.

SPORE STAIN: A Safe Spore Stain for Class Use, May, H. G. Stain Technology, July, 1926, i, No. 3, p. 105.

1. Make film and fix by heat as usual.
2. Cover with small amount of 5 per cent chromic acid.
3. After thirty seconds add about twice as much concentrated ammonia as there is chromic acid on the slide. Allow to act about two minutes.
4. Rinse with tap water.
5. Steam with carbol fuchsin for two or three minutes.
6. Rinse.
7. Destain with one per cent sulphuric acid for fifteen to thirty seconds.
8. Rinse again and flood slide with the tap water.
9. Add to this a few drops of Loeffler's methylene blue and allow to stain for ten to thirty seconds.
10. Rinse, blot, dry and examine.

The dilution of the methylene blue is necessary because the pre-treatment increases the staining properties of the cells so much that a good methylene blue overstains when used in concentrated form even if rinsed off a second or so after it is put on the slide.

The results with this method will not always be equally good, as a lack of sufficient destaining may leave some vegetative cells red or an overstaining with methylene blue will make the cells opaque and even form a heavy film over some of the spores, but the spores will always be stained and clearly visible. The average student has no more difficulty with this method than with the Gram stain and usually obtains more reliable results.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Medical Laboratory Methods and Tests**

THE purpose of this book is to present, for the use of the physician, the chemical, microscopic, and other examinations which may be made in the office laboratory.

Many of the procedures are, quite naturally, those in vogue in Europe, although the work of American investigators is not neglected, and the work should serve very well for the purpose for which it is intended.

Forensic Medicine and Toxicology†

THE increasing importance of medical evidence and the fact that any physician may find himself called as a witness renders some knowledge of medicolegal procedure a necessity. Such a survey is presented in this ninth edition of Buchanan's work.

The book is divided into two sections, the first concerned with forensic medicine, and the second with toxicology.

While the legal procedures are those of the English courts, the book should prove useful to American readers because of its clear presentation of the subject.

A Statistical Survey of Three Thousand Autopsies‡

A REPORT of a study of the postmortem examinations performed during the years 1900-1923. None but fairly complete autopsies are included and the material surveyed is exhaustively classified and cross-indexed.

The report is divided into nineteen sections, each concerned with particular conditions and is embellished with numerous tables and charts.

It illustrates anew the striking importance of congenital syphilis as a cause of infant mortality (112 of 307 cases).

The first three sections are concerned with a general survey, the remainder discussing the conditions found in seriatim.

The relative frequency with which various conditions were encountered is shown below:

*Medical Laboratory Methods and Tests. By H. French, Physician, Guy's Hospital, and T. Nuthall, Assistant, Guy's Hospital. Cloth. Pp. 246. 62 illustrations and 3 colored plates. Price $2.50. Chicago Medical Book Co.

†Forensic Medicine and Toxicology, ed. 9 (Buchanan), revised, and enlarged by J. E. W. McFall, Professor in Forensic Medicine and Toxicology, University of Liverpool. Cloth. Pp. 445. 57 illustrations. Price $5.00. William Wood and Co.

‡A Statistical Survey of Three Thousand Autopsies. By Wm. Opuls, Department of Pathology, Stanford University. Paper. Pp. 370. 16 charts. Price $2.50. Stanford University Press.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

Tuberculosis	64 %
Septic infections	42 %
Arteriosclerosis-nephritis	37.5 %
Gonorrhea-syphilis	31 %
Tumors	30 %
Malformations	14.5 %
Miscellaneous lesions	40 %
Calculi	11 %
Alcoholism-drug addiction	9.5 %
Injuries-poisonings	10.6 %
Acute infections	6.6 %
Cirrhosis-anemia	10 %
Diseases of metabolism	13.8 %
Animal parasites	1.75%

The enormous amount of material thus gathered and minutely studied should be invaluable to pathologists and those interested in disease.

*Diseases of Children**

IN HIS prefactory remarks Cameron defines his handbook as one that deals briefly with the fundamental principles involved in the application of the fundamental and primary conceptions relating to pediatrics. This presentation makes no pretense of being a complete textbook, but on the contrary, stresses its message to medical readers in a most impressive, brief, and practical manner. The book is more or less devoid of theoretical discussions, laboratory procedures, or microscopic and gross pathology. It presents in a practical manner the salient features of certain common disorders met with in infancy and childhood, as viewed and interpreted through the eye of the pediatric clinician. The wealth of condensed and common-sense observations are presented in a somewhat similar manner as found in his splendid and famous book, *The Nervous Child.* Cameron deplores the lack of a more profound and intensive teaching of pediatrics in the routine medical curricula of schools and colleges, and when one takes into consideration that approximately 30 per cent of the average physician's patients are within the age limits of infancy and childhood, certainly this criticism is amply justified. The subjects of the thirteen chapters were chosen to present the most important everyday problems that confront the physician in the routine course of his medical duties, and it is with this view that Cameron's vision and broad knowledge are interpreted within the pages of this small textbook.

The chapters are captioned as follows:

The Study of Disease in Childhood.
Diseases of the Newly Born: Congenital, Traumatic, and Infective.
Breast Feeding and Its Management.
Sleeplessness and Nervous Unrest in Infancy.
Catarrhal Infection.
Vomiting in Infancy.
Diarrhea in Infancy.
Constipation.
Inherited Predisposition to Disease.
Conduct and Management and Their Effect Upon Health.
Some Surgical Operations Upon Children.
Backwardness and Convulsions in Infancy.
Diet.

As a medical handbook the features and procedures that are chosen are so well and clearly presented that it is indeed worth while to absorb Cameron's broad clinical experiences in the several chapters of this booklet.

*Diseases of Children. By H. C. Cameron, Physician in Charge, Department for Diseases of Children, Guy's Hospital. Cloth. Pp. 199. Oxford University Press, 1926.

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EDITORIALS

The Prophylaxis of Acute Infections

QUARANTINE as a prophylactic measure is, perhaps, one of the oldest means utilized in the prevention of disease, as it is mentioned in Biblical writings, referred to in some of the earliest known hieroglyphic records and, perhaps, under the guise of "taboo" may be recognized among the antiquities of primitive peoples.

Originally applied without distinction to disease in general, the manner and duration of quarantine has undergone various modifications in modern days in accordance with the evolution of understanding of the etiologic agents and the manner of their dissemination in various diseases, and recent studies suggest some further modification of the value of the method as applied to certain specific infections, such as scarlet fever.

Holst[1] has recently published a study of the relation of quarantine to the incidence of scarlet fever in Norway which, while not final in its conclusions, at least opens up an interesting vista and suggests the advisability of the collection of similar and more extensive data in other countries.

In Norway scarlet fever is treated largely by isolation in the home or hospital, and a study of the incidence of the disease reveals the lowest inci-

dence where living conditions and the absence of hospitals have always made the fight against the disease difficult, and the highest incidence where the facilities are the best.

That this is not due merely to incompleteness of notification is shown by the fact that in Bergen, representing the first situation, but where notification is as effective as in Oslo, representing the second situation, the incidence is much lower than in the last named city.

Statistics quoted by Holst covering 21 cities and the country district for the years 1880 to 1919 show that it is impossible to prove statistically that the disease can be eradicated by isolation, but the same statistics also show that, whereas in the early eighties scarlet fever was one of the most important causes of death, in later years it becomes relatively unimportant.

What is the relation of quarantine, if any, to this fact?

According to Chapin,[2] isolation by the segregation of grave cases due to a virulent strain of the virus is the reason for the lessened mortality, but Holst, from a comparison of the statistics of Norway and Sweden, points out that the decline in the Norwegian mortality curve was most marked when hospital isolation was still very incomplete and in those parts of the country where it was most difficult to control the spread of the disease. Holst, therefore, cannot agree with Chapin's conclusion and maintains that the incidence of scarlet fever is dependent upon conditions still unknown and that it has not been perceptibly influenced by quarantine measures.

He comments upon these facts:

1. Practical experience shows that scarlet fever is frequently transmitted by contact.

2. The not infrequent absence of any source of infection suggests the possible existence of a carrier.

3. There is no definite knowledge as to the period of infectivity.

All these facts in sum affect the rationale of quarantine and modify its duration, experience showing that some convalescents are harmless after three weeks while others are still dangerous after six weeks.

The quarantining in hospitals of grave cases with mild cases which are thus exposed to grave complications has also been objected to on this ground, and the undoubted fact that many mild cases are unrecognized until the period of desquamation has led, in Bergen since 1910, to the exclusion from school of contacts for a period of only five days—a procedure apparently without effect upon the incidence of the disease.

In two Norwegian cities—Bergen and Trondjhem—scarlet fever has been treated since 1910 without isolation and without disastrous results. Houses are placarded and prompt notification is insisted upon, and while the other children are kept from school for five days they are allowed to play in the street; after desquamation is completed the patient's room is cleaned, but no further precautions are taken. A comparison of the cases per thousand in Bergen and in Oslo, where strict hospitalization is practiced, shows a constantly lower rate for the former city from 1900 to 1922.

Holst concludes, therefore, that the abolishment of isolation has had no influence upon the incidence of scarlet fever, but he is uncertain as to the

effect upon the case mortality. He believes that it is essential to keep in touch with the disease because malignant or fulminating characteristics may appear at any time.

Reference has been made to the variability of the period of infectivity during convalescence from scarlet fever and the studies of Gordon[3] upon the presence of hemolytic streptococci in the upper respiratory tract of scarlet fever convalescents are of distinct interest in this connection.

The apparently normal convalescent carrier is, obviously, of essential importance but, despite this fact, little is known of this type. Gordon comments upon the variability of opinion as to the period of infectivity and the great value of a method of cultural control in scarlet fever similar to that in diphtheria and reports his studies of 200 cases over a period of eight months from which he draws the following conclusions:

The severity of the disease definitely influences the time that hemolytic streptococci remain on the mucous surfaces of the upper respiratory tract, being less for mild than for moderately severe, and still less than for severe cases.

The administration of antitoxin apparently influenced the frequency of positive cultures, the incidence being 38 per cent as opposed to 64 per cent in cases not receiving serum.

Age and sex were without apparent effect while complications definitely influenced the incidence of positive cultures, serum-treated cases without complications having 32 per cent, and those with complications 51 per cent of positive cultures, and the rate for control cases being 58 per cent without and 75 per cent with complications.

Gordon believes, therefore, that complications account for chronic carriers and that a severe uncomplicated case is safer for release than a mild case with complications.

In positive cases the organisms are most commonly found in the throat only; about one-third show organisms in both throat and nose; and only rarely is only the nose positive. The highest percentage of positive cultures occurs in the age group in which complications are most frequent.

From his studies Gordon believes that consideration should be given to the practicability of a revision of the existing regulations for isolation in scarlet fever and that some variation in time be governed by cultures for scarlet fever streptococci.

As a corollary to these studies Harmon and Perkins[4] have analyzed the data concerning the relation of latitude to the seasonal occurrence of scarlet fever, and also of diphtheria and measles in the United States and in Australia, emphasizing the time of year in which the maximum and minimum incidence of these diseases occurs, rather than the total number of cases.

Their statistics covering the years 1913 to 1924, inclusive, show that, in the United States, November, May, and January are the months of maximum, and July, September, and August, the months of minimum incidence for the diseases in question, the peak of seasonal prevalence being from one to three months earlier in the South than in the North, these tendencies being observed in years of both low and high prevalence.

While the analysis of their material suggests that latitude has an effect upon the seasonal distribution of the prevalence of these diseases, the degree to which temperature, moisture, sun's rays, or other factors are concerned, awaits the accumulation and analysis of further data which, it is to be hoped, may be furnished eventually through the labors of the League of Nations.

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—*R. A. K.*

The Doctor's Bookshelf

THE doctor, of all men, must be a lover of books, for reading, says one of the ancients, "maketh a full man."

He must, of course, be a reader and a student of medical texts because medicine is a changing science always in a state of evolution, the theories of today being proved or disproved tomorrow.

The tendency of his medical library naturally will be to concentrate itself around the subjects which constitute his predominant interest but there will be, if he is to be well-grounded and well-rounded in his profession, a judicious sprinkling also of those works which include allied subjects and those contiguous to and inseparable from his particular interest.

The necessity for this is obvious for his subjects are human beings, and human beings are not hearts, lungs, stomachs, and various other separate anatomic entities, but complex and intricate beings composed of innumerable structures welded into one inseparable and interlocking whole.

There will be, first of all, works on the causes and mechanism of disease production, and these should show honorable scars of frequent use, for, if one were restricted to one field of study only, it would be most valuable to know the pathology, that is, the mechanism whereby functional disruption, which is disease, occurs.

The care with which his patients are studied and their symptoms interpreted will be reflected in that part of the bookshelf which pertains to diagnosis and the means and methods whereby it may be achieved. In that part of the library pertaining to treatment the most valuable sources of information will be found in the doctor's case records which are really, after all, the tomes in which are writ the lessons of experience.

It is better to have a small library and to read it than to have a large one mainly to look at.

Every man interested in his work will, sooner or later, begin the collection and filing of pertinent reprints and data. The completeness with which this is done will reflect the study and survey of the current and sometimes ephemeral literature of journals. The manner of its doing is a matter of personal preference but should be devised to render the data accessible.

Doctors are busy men and many there are who "have not time to read." This is hardly a valid excuse. A famous Practice of Medicine is said to have been written largely in the short interval between breakfast and the morning office hours. There is always time to do what one wants to do.

It is quite difficult to read a textbook from cover to cover, and doubtful if the information absorbed is worth the labor; but many a case has a puzzling feature, a phase concerning which recollection is faulty and which may be looked up profitably, even when one is busy. And such a practice leads to much reading in the long run.

It is impossible, of course, to carry the contents of ones bookshelf in one's mind, but it is possible to know, without haphazard search, where to go for specific information and how to utilize it when it is found.

Books must be read to be useful. A medical library, unlike others, is ephemeral. The fame of the author is not always a true index to the true worth of his book but, in the main, it warrants at least an examination of the work. It is one thing to have knowledge and another to have the ability to impart it.

Monographs are sometimes of more present value than ponderous sets, sometimes, also, more likely to be read.

It is well not to buy solely because the work is new; be sure the subject is of sufficient interest to tempt you to read, and the author capable of his subject.

At least one authoritative work on the history of medicine should be included and read.

The medical library will, of course, be a part of the workshop and its armamentarium and not the least valuable.

There will be, without doubt, other books: the classics, and those concerned with particular interests and special hobbies.

The doctor's interest in and choice of literature will prove a shrewd index to his personality. An interest in literature is allied to an interest in his profession for he must be a student of human nature, and in the classics he will find it dissected and portrayed; he must appreciate good writing if he is to do any; even a case history to be truly valuable should be well written, and in his books he will find examples to admire and profit by.

The doctor must know how to express himself if he is to impart his thoughts to others by word or pen, and from his books and reading he will draw his vocabulary and learn how to marshal his words and sentences into companies and divisions to carry the trenches of opposition or ignorance by irresistible assault.

And, in the learning, when the day's work is done and the doctor sits in the quiet shadows, living, perhaps, in other days, he will find pleasure, relaxation, and contentment, and in the companionship of his bookshelf find his other self, coming forth renewed in mind and so refreshed in body, with the facets of his mind reflecting old impressions and ready for the new.

—*R. A. K.*